Effects of Acute Nutritional Interventions on Athletic Performance

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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 nor material which to a substantial extent has been accepted for the award of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made.

Tom Vandenbogaerde

Date:

ABSTRACT

There have been few studies of the effects of nutritional strategies in training and competition settings in elite athletes. This thesis represents four studies that were performed to investigate the effect of specific acute supplementation protocols on performance and/or recovery from exercise. Studies 1-3 were experimental investigations of the recovery and/or performance effects of carbohydrate, carbohydrate-protein or caffeine supplements in elite swimmers. Study 4 was a meta-analytic review of the effects of acute carbohydrate supplementation on endurance performance.

In Study 1, we have provided some evidence that consuming carbohydrate during and carbohydrate-protein immediately after a 2-h high-intensity swim session induces better recovery in plasma creatine kinase and salivary IgA compared with consuming water during exercise and carbohydrate-protein immediately after, and compared with consuming only carbohydrates during and immediately after exercise. These effects may indicate reduced muscle damage and better mucosal immunity in the upper respiratory tract. The inclusion of protein in the carbohydrate supplement also reduced inflammatory responses. As demonstrated in the meta-analysis (Study 4), consuming carbohydrate and carbohydrate-protein supplements during exercise can have large benefits in endurance performance: The best supplement inferred from the analysis consisted of the best regime derived from the analysis consisted of ingesting a ~3-10% carbohydrate-plus-protein drink providing ~0.7 $g \cdot kg^{-1} \cdot h^{-1}$ glucose polymers, ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ fructose and ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ protein in multiple boluses before and during exercise. Caution is required to extrapolate the results of the meta-analysis to short-duration exercise, because the meta-analysis included only one study with exercise duration <25 min. In Study 2, we found possible performance impairments in the last step of a 7x200-m step test (change in performance time 0.9%; 90% confidence limits ±1.1%) and in a 100-m time trial (0.1%; ±0.6%) with ingestion of a carbohydrate-protein supplement. In Study 3, we have provided some evidence that ingesting ~100 mg caffeine 75 min before training or competition time trials enhances performance in elite swimmers by ~1.3%. This

intervention was part of a methodological investigation of a novel application of mixed linear modeling for monitoring athletic performance.

Through this PhD research, we have demonstrated clear performance and recovery effects with specific acute supplementation protocols in elite swimmers. We have provided a novel approach to investigate effects of treatments in elite athletes, and we have demonstrated large effects with carbohydrate supplementation regimes in endurance exercise in an innovative meta-analytic review. We encourage athletes, sports scientists and coaches to estimate magnitudes of effects of treatments and individual responses to treatments using linear modeling of performance times.

PREFACE

Thesis Rationale

Researchers have determined many ways to enhance performance and recovery from exercise through nutrition, but there have been few studies of effects in training or competition with elite athletes. The primary focus of this PhD was to assess the effects of specific nutritional interventions on performance and/or recovery from exercise in elite swimmers.

Soon after my arrival in New Zealand, Jan Cameron, at that time the head coach of the New Zealand High Performance Swim Program, expressed an interest in improving nutritional protocols for recovery from exercise. We therefore investigated the acute effects of various nutritional protocols on recovery from exercise in the first project of the PhD. Of topical interest was the inclusion of protein in a carbohydrate supplement. Will Hopkins had created the exciting opportunity of collaborating with the Horticulture and Food Research Institute of New Zealand. This collaboration allowed us to evaluate concentrations of a wide range of salivary and blood markers associated with immunity and recovery (for review see Moreira et al., 2006). As a result of this research, we began promoting the ingestion of carbohydrate and carbohydrate-protein supplements during exercise for better recovery. However, we were concerned about the performance effect of this strategy in high-intensity swim sets and competition. Although previous research had demonstrated that carbohydrate and carbohydrate-protein supplements enhance performance in prolonged cycling and running, there had been no research investigating the performance effects of these supplements in short-duration swim exercise. This issue was addressed in the second project with a carbohydrate-protein supplement similar to a supplement suggested in a conference abstract (Seifert & McKenzie, 2007) to enhance performance in high-intensity swim sets.

In the preparation for the trials for the 2008 Olympic Games, the athletes of the New Zealand High Performance Swim Team asked me whether they should take caffeine to enhance performance in competition. Although many previous studies had addressed the effect of caffeine on endurance performance (for review see Warren, Park, Maresca, McKibans, & Millard-Stafford, 2009), its effects on performance in short-duration

exercise and sprinting in well-trained athletes were less clear. In particular, a recent conference abstract showed little effect of a low dose of caffeine on 100-m swim performance in elite athletes (Burke, Anderson, & Pyne, 2006). We addressed this issue in the third project. We scheduled a caffeine cross-over intervention in the swim program and estimated the effect of ingesting 5 $mg \cdot kg^{-1}$ caffeine on performance. This intervention was made part of a methodology paper in which we have described a novel approach for monitoring acute effects of training and other interventions on athletic performance. This approach allows to some extent the quantification of individual responses, which is an important but often neglected aspect in experimental research.

The fourth and final project comprised a meta-analytic review of the effects of acute carbohydrate supplementation on performance. This review does not encompass all aspects of the PhD, but was developed from the literature reviews of the two first studies. Although research on the effects of carbohydrate supplements is comprehensive (for reviews see Bosch, 2007; Jeukendrup, 2008), there has been no published meta-analytic review. In this review, we have devised ways to overcome difficulties with producing a common metric for effects on performance in various exercise protocols and with poor reporting of inferential statistics. We successfully combined the magnitude of more than a hundred study-estimates into a meta-analyzed effect, with moderating effects for various study and supplement characteristics.

Thesis Organization

This thesis consists of five chapters. The references for each chapter are at the end of the chapter and are collated at the end of the thesis. Chapters 1-4 are original investigations. Chapter 1 has been submitted to the *International Journal of Sport Nutrition and Exercise Metabolism*. Chapter 2 has been submitted to the *International Journal of Sports Physiology and Performance*. Chapter 3 has been accepted for publication in *Medicine and Science in Sports and Exercise*. Chapter 4 is the main review of the literature for the thesis and has been submitted to *Sports Medicine*. Chapter 5 comprises a general summary in which practical applications of this research are discussed.

The appendices are presented in chronological order of their development in the PhD. Appendix A contains the poster for "Acute Effects of Nutritional Supplementation on Recovery from Training in Elite Swimmers", which won the award for best poster presentation at the 2007 Conference of Sports and Exercise Science New Zealand. Appendix B is the paper "Development and Validation of a Sensitive Immunoassay for the Skeletal Muscle Isoform of Creatine Kinase" that I have co-authored and that has been published in the *Journal of Science and Medicine in Sport*. I have not included this study as one of my PhD chapters, as I was a minor author. Appendix C comprises the Powerpoint presentation of "Acute Effects of a Carbohydrate-Protein Sports Drink on Performance in Swimmers" that was given at the XXX FIMS World Congress of Sports Medicine in Barcelona 2008. Appendix D contains data that we have removed from the manuscript of the second study, "Acute Effects of a Carbohydrate-Protein Supplement on Performance in Swimmers". These data on subjective ratings of muscle soreness and coping with exercise were obtained the day after the performance test and were removed because of our concern that the data were confounded by the knowledge of performance in the test. Appendix E is the poster of "Monitoring Acute Effects on Athletic Performance with Mixed Linear Modeling", that Will Hopkins presented on our behalf at the annual conference of the European College of Sport Science in Oslo 2009. Appendix F comprises scales that were used to measure muscle soreness and coping with exercise in two of the experimental studies (Chapters 1 and 3). Appendix G contains the abstract for "A Competition-Based New Research Design to Assess an Intervention Affecting Performance of a Squad of Elite Athletes"; I will present this study at the XIth International Symposium on Biomechanics and Medicine in Swimming in Oslo 2010. Apart from the fact that this study has not yet been written up, we have not included it as one of the thesis chapters, because it does not specifically address aspects of effects of nutritional strategies on performance or recovery from exercise.

The attached CD contains data, statistical analyses and relevant information from ethics applications for the studies presented in Chapters 1-4.

Publications and Conference Presentations Arising from this Doctoral Thesis

1. Peer-reviewed published and accepted articles

Vandenbogaerde TJ, Hopkins WG. Monitoring Acute Effects on Athletic Performance with Mixed Linear Modeling. *Medicine and Science in Sports and Exercise*. 2010; 42:1339-1344. (Author contribution percentages: TV, 80%; WH, 20%)

Lo KR, Hurst SM, Atkinson KR, Vandenbogaerde TJ, Beaven MC, Ingram JR. Development and Validation of a Sensitive Immunoassay for the Skeletal Muscle Isoform Creatine Kinase. Journal of Science and Medicine in Sport. 2010; 13:117-119. (TV, 10%)

2. Articles submitted to peer-reviewed journals

Vandenbogaerde TJ, Hopkins WG, Hurst SM. Effects of Acute Dietary Interventions on Markers of Recovery in Elite Swimmers. *International Journal of Sports Physiology and Performance*. 2010; submitted, October. (TV, 80%; WH, 10%; SH, 10%).

Vandenbogaerde TJ, Hopkins WG, Talbot, ST, Ingram JR. Acute Effects of a Carbohydrate-Protein Sports Drink on Performance in Swimmers. *International Journal of Sports Physiology and Performance*. 2010; submitted, October. (TV, 80%; WH, 15%; ST, 2.5%; JI, 2.5%)

Vandenbogaerde TJ, Hopkins WG. Effects of Carbohydrate Supplements on Endurance Performance: a Meta-Analysis. *Sports Medicine*. 2010; in revision, October. (TV, 80%; WH, 20%)

3. Conference presentations

Vandenbogaerde TJ, Hopkins WG, Hurst SM. Acute Effects of Nutritional Supplementation on Innate Immune Response in Elite Swimmers. *New Zealand Sports Medicine & Science Conference Programme and Collected Abstracts, Hamilton, New Zealand*. 2007; p. 104. This poster was awarded the prize for Best Poster Presentation.

Vandenbogaerde TJ, Hopkins WG, Talbot, ST, Ingram JR. Acute Effects of a Carbohydrate-Protein Drink on Performance in Swimmers. *Archivos de Medicina del Deporte*. 2008; 128: 478.

Vandenbogaerde TJ, Hopkins WG. Monitoring Acute Effects on Athletic Performance with Mixed Linear Modeling. Proceedings of the European College of Sport Science Conference, Oslo, Norway, 2009. The abstract is available on http://www.ecss-congress.eu/OSLO2009/images/stories/Documents/BOAOSLO0610bContent.pdf (p. 415).

Vandenbogaerde TJ, Hopkins WG, Pyne DB. A Competition-Based New Research Design to Assess an Intervention Affecting Performance of a Squad of Elite Athletes. Proceedings of the XIth International Symposium on Biomechanics and Medicine in Swimming, Oslo, Norway, 2010. The abstract is available on *http://www.nih.no/Upload/BMS2010/Documents/BM2010_Program_Abstracts_final_lowres.pdf* (abstract 0-073).

Vandenbogaerde TJ, Hopkins WG. Effects of Carbohydrate Supplements on Endurance Performance: a Meta-Analysis. Proceedings of the European College of Sport Science Conference, Antalya, Turkey, 2010. The abstract is available on *http://www.ecss2006.com/asp/congress/ScPro1AbstractText.asp?MyAbstractID=797* (abstract 797).

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Many people have helped me realize this PhD. My parents have given me the freedom to explore and develop my career path, and have given me financial and emotional stability for the goals I wanted to achieve. My deepest thanks go to them. You have been far away, but I have always felt your presence and support. You are beautiful, special people, and I am proud to be your child.

Will Hopkins has been the best supervisor and mentor I could have wished for. Brilliant, humble, patient and down to earth are only few of his many qualities. For such a famous Professor, I expected someone in a suit whom only few people would be allowed to address with “Will”. Nothing is less true. When we first met, he arrived at my flat in

jeans, sport shoes, a woolen sweater and wild hair. I have always felt comfortable around him. The thing with Will is that you always know when you do right and when you do wrong. Will, I have learnt so much from you, not only in science and research, but also as a person. This PhD is as much yours as it is mine. I can't thank you enough.

Jan Cameron is another special person who needs special acknowledgement. Jan is not only a successful coach, manager and director, she is also a second mother to many, a mother with a great heart. She has heart for swimming, she has heart for people, and she will fight for her people and beliefs. Her determination and drive are difficult to be matched. Jan, I have learnt a lot from you and you have given me opportunities that billions of people dream of, the most amazing of which being part of the Olympic Team for Beijing. Thank you so much, for everything!

My best thanks also go to Thomas Ansorg. I have been working with Thomas at poolside since the day I arrived in New Zealand. He has the most amazing stories to tell. If you ever get the chance to have a casual beer with Thomas, ask him about Africa. You might end up having a lot more beers! He has fascinating stories to tell, and this translates in his coaching. Thomas is a very driven and determined coach. Thomas, I have learnt many, many things from you, thank you!

I would also like to thank the swimmers. You have the talent and determination many people are jealous of. I wish you success at the highest level. Yes, you can, and you know how to. Be determined, be focused, give it your all, and although the path may seem hard at times, never give in!

I also acknowledge the Horticulture and Food Research Institute of New Zealand for their partnership, for their support and sponsorship, and for teaching me lab skills. Sue Hurst, John Ingram, Kim Lo and Kelly Atkinson, thank you!

Nestlé and Musashi have been very generous with providing Musashi supplements for our first study. They have helped us and the swim program a great deal! Maurice Gunnell and Marshal Adam, thank you!

Special acknowledgement also goes to Swimming New Zealand, the New Zealand Academy of Sport, and AUT University. Jan, Clive, Martin, Pete, Will, Patria, you have created this opportunity. I am very, very grateful!

Thanks to my mentors in Belgium. Prof Jean Pannier, Prof Jacques Bouckaert, Prof Paul Coucke, Prof Wim Derave and Filip Roelandt, I would not have started this PhD without the inspiration and support you have given me.

Then last, but not least, my best friend in New Zealand, Rachel. I am sure there are only few people around with as much patience and understanding as yours. At work, I have always been strong, but Rachel has seen me in various conditions, and she has always been there for me. Rachel, you are very unique, and you are very precious to me, you will always be.

To all of the people I have named above: this PhD has been a team effort–I could never have achieved this dream without you!

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Warren, G. L., Park, N. D., Maresca, R. D., McKibans, K. I., & Millard-Stafford, M. L. (2010). Effect of caffeine ingestion on muscular strength and endurance: a meta-analysis. *Medicine and Science in Sports and Exercise, 42*, 1375–1387.

CHAPTER 1

Effects of Acute Dietary Interventions on Markers of Recovery in Elite Swimmers

Abstract

Purpose: To compare the effects of three acute dietary supplementation protocols on markers of muscle damage (plasma creatine kinase), oxidative stress (plasma protein carbonyls), adrenocortical function (salivary cortisol), and immune function (plasma and salivary cytokines; salivary IgA). We also assessed effects on subjective ratings of muscle soreness and coping with exercise. ***Methods:*** In a double-blind randomized crossover, nine elite swimmers (6 females and 3 males; age 18-24 y) were subjected to three supplementation protocols in a 2-h high-intensity swim-training session at intervals of one week: a carbohydrate beverage during and immediately after exercise (CC); a carbohydrate beverage during exercise with a carbohydrate-protein mixture immediately after exercise (CP); and a non-caloric placebo during exercise with a carbohydrate-protein mixture immediately after exercise (WP). Recovery was assessed by comparing means of variables 12 h after exercise. ***Results:*** Creatine kinase was lower with CP relative to CC (qualitative outcome small; ratio 0.83; 90% confidence limits ×/÷1.4) and WP (small; 0.79; ×/÷1.3). Salivary cortisol was lower with CC relative to CP (moderate; 0.37; ×/÷2.5) and WP (moderate; 0.35; ×/÷2.1). Salivary IgA was higher with CP relative to CC (small; 2.1; ×/÷2.3) and WP (moderate; 3.4; ×/÷2.2). Differences in protein carbonyls, cytokines and subjective ratings were trivial, small or unclear. ***Conclusion:*** Acute dietary supplementation protocols had substantial effects on some markers of recovery after high-intensity swim exercise, but the functional relevance of the effects is uncertain.

Keywords*:* athlete, carbohydrate, immunity, protein

Introduction

The use of carbohydrate and carbohydrate-protein supplements to enhance recovery from training in athletes is a current research issue. Carbohydrate-protein supplements during or immediately after exercise reduce muscle soreness and plasma creatine kinase, indicating a reduction in muscle damage.[1-5] Carbohydrate before and during exercise attenuates the exercise-induced increase in cortisol (for review see Moreira et al.[6]), a marker of adrenocortical function that could be important in recovery. Effects of carbohydrate and protein supplements on immune function, another potential marker of recovery, remain unclear: several groups have reported attenuated increases in immune signaling hormones (cytokines) with carbohydrate supplementation before or during exercise, but others have failed to show any significant effect (for review see Moreira et al.[6]). The volume of fluid ingested during exercise affects salivary IgA, a marker of mucosal immunity in the upper respiratory tract, but there is little effect of carbohydrate on this marker.[6,7]

More research is necessary to fine-tune dietary supplementation aimed at enhancing recovery from training. In this study we have compared the effects of three acute dietary interventions during and immediately after a high-intensity training session on markers of recovery in elite swimmers. One intervention, consisting of carbohydrate supplementation during and immediately after exercise (CC), was iso-caloric with another intervention, consisting of carbohydrate supplementation during exercise and carbohydrate-protein immediately after exercise (CP). These interventions were included to determine the effect of protein in the post-exercise supplement when carbohydrate is consumed during exercise. The third intervention consisted of water during exercise and carbohydrate-protein immediately after exercise (WP), because training in this manner may cause a beneficial "recycling of glycogen".[8] Owing to concerns about the effects of blood loss on performance, we limited our focus to recovery for the next training session by measuring marker concentrations in samples taken immediately after a training session and immediately before the next training session, 12 h later.

Methods

Subjects

Originally 16 athletes took part, but blood and saliva samples were analyzed only for the 9 athletes who were present on all testing occasions. All subjects gave written informed consent as required by the AUT University ethics committee, which approved this study. Subject characteristics are shown in Table 1. Subjects (6 females and 3 males; age 18-24 y) were highly trained swimmers competing at national (n=5) and international (n=4) level. The swimmers were performing a similar training program consisting of two 2-hour swim sessions each day, except for Wednesdays and Saturdays with only a morning session and Sundays with no session. Sessions started at 6.30 am and 4 pm. The interventions took place on three consecutive Thursday afternoons in a period of medium-volume training (60-70 km per week). The swimmers had abstained from any resistance training for two days prior to each intervention. Dietary carbohydrate and protein intakes were calculated from a 3-day diet diary that was completed two weeks before the start of the study. Five of the swimmers consumed only water during training sessions covered by the diaries; the others consumed ~500 ml per session of sports drinks containing ~7% carbohydrate.

Table 1 Subject characteristics

	Females (n=6)	Males (n=3)
Age (yr)	22 ± 3	21 ± 3
Height (cm)	175 ± 4	187 ± 1
Body mass (kg)	65 ± 5	78 ± 5
Dietary carbohydrate intake (% total energy intake)	51 ± 10	54 ± 3
Dietary protein intake (% total energy intake)	16 ± 4	19 ± 7
Recent performance (% world record)	94 ± 4	94 ± 2

Data are means ± SD.

Diet Control

All subjects consumed a carbohydrate beverage during exercise and a carbohydrate-protein beverage immediately after exercise in Wednesday and Thursday am sessions pre intervention, and in the Friday am session post intervention. The athletes were advised to consume 500 ml fruit juice together with lunch before each intervention, and to have the

same diet before each intervention. A high-carbohydrate dinner differing only in the choice of meat (chicken or fish) was provided 45 min after each intervention session.

Study Design and Supplementation Protocols

The supplementation protocols and the timeline of the study are shown in Figure 1. The design was a double-blind randomized crossover trial of three supplementation protocols (CC, CP and WP). Carbohydrate was a combination of glucose, maltodextrin and fructose; protein was a combination of essential amino-acids. The interventions took place in three Thursday afternoon high-intensity ~6-km swim training sessions with a one-week wash-out in between. To gauge recovery, saliva and venous blood samples were taken immediately after exercise and 12 h later, immediately before the Friday morning swim training session.

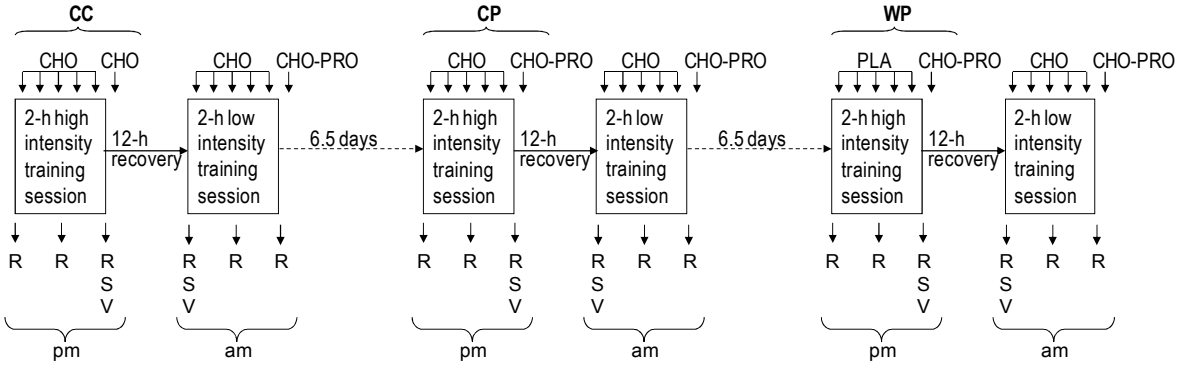
Subjective Ratings

Before, during and immediately after exercise in both the intervention session and the session next morning, the athletes gave subjective ratings of leg soreness and arm soreness between 0 and 100 on a 21-point scale anchored throughout with descriptors ("no pain at all" through "unbearable").[9] The three values for each training session were averaged for analysis of the overnight change. The scale and questions were adapted for ratings of coping with exercise (by changing the wording to "how will you cope", "how are you coping", and "how did you cope" for the ratings before, during and after exercise, respectively). Reliability expressed as error of measurement on the 0-100 scale (and retest correlation) calculated from the morning values of the subjective ratings were as follows: coping with exercise, ±7.1 (0.74); leg soreness, ±7.0 (0.86); and arm soreness, ±6.9 (0.77).

Saliva and Blood Sampling

Subjects received sugar-free chewing gum and a collection tube to sample 4 ml of saliva. None of the athletes experienced difficulty in providing the sample within 5 min. All saliva samples were immediately stored at -20°C for later analysis. Blood samples (8 ml) were taken from a median cubital vein by venepuncture, and collected into Vacutainer tubes by experienced phlebotomists. Blood was spun at 2000 g for 10 min

within 1 h of sampling. The plasma obtained was immediately stored at -80°C for later analysis.



CC: carbohydrate beverage during exercise (6.6 g·100ml^{-1} carbohydrate; females 66 g; males 88 g) and immediately after exercise (10 g·100ml^{-1} carbohydrate; females 53 g; males 64 g)

CP: carbohydrate beverage during exercise (6.6 g·100ml^{-1} carbohydrate; females 66 g; males 88 g) with carbohydrate-protein mixture immediately after exercise (8.0 g·100ml^{-1} carbohydrate; females 40 g carbohydrate and 13 g protein; males 48 g carbohydrate and 16 g protein)

WP: non-caloric placebo during exercise with carbohydrate-protein mixture immediately after exercise (8.0 g·100ml^{-1} carbohydrate; females 40 g carbohydrate and 13 g protein; males 48 g carbohydrate and 16 g protein)

Figure 1 — Study design and supplementation protocols. The order of CC, CP and WP interventions was randomized. CHO, carbohydrate beverage; CHO-PRO, carbohydrate-protein beverage; PLA, non-caloric flavored water placebo; R, ratings of coping and soreness; S, saliva sample; V, venous blood sample.

Assays

Protein carbonyls were measured by the method of Levine.[10] Other salivary and blood markers were assayed with commercial kits as follows: plasma creatine kinase using spectrophotometry on a Hitachi 747 analyzer and reagents from Boehringer Mannheim (Mannheim, Germany); salivary cortisol using radioimmunoassay (Diagnostic Systems, Webster, TX); cytokines using ELISA (R&D Systems, Minneapolis, MN); and salivary IgA using EIA Kit (Alpco Diagnostics, Windham, NH). Reliability expressed as factor error of measurement (and retest correlation) calculated from the morning values of the markers were as follows: creatine kinase, ×/÷1.4 (0.82); protein carbonyls, ×/÷1.3 (0.53); salivary cortisol, ×/÷2.6 (0.51); plasma IL-1β, ×/÷1.5 (0.96); plasma IL-6, ×/÷2.1 (0.95); plasma IL-8, ×/÷2.5 (0.93); salivary IL-6, ×/÷2.0 (0.80); salivary IL-8, ×/÷1.6 (0.67); and salivary IgA, ×/÷2.3 (0.63).

Statistical Analyses

Statistical analyses were performed separately for each marker concentration and subjective rating using an Excel spreadsheet for crossovers.[11] To quantify overnight recovery, we calculated differences in concentrations and ratings 12 h after exercise (am) between the three pairs of interventions. We also measured the differences between carbohydrate and water consumption during exercise on marker concentrations immediately after exercise (pm) and on mean subjective ratings with exercise (pm) by subtracting the average of the values for CC and CP from the value for WP. Finally, we investigated the relationship between values in plasma and saliva for IL-6 and for IL-8 by calculating the correlation coefficients for all 54 observations (9 subjects, 6 sessions) after log transformation; we performed similar analyses for the 54 observations of the subjects' deviations from their means. Confidence limits for the correlation coefficients were calculated with a spreadsheet[12] by using the minimum sample size of nine independent observations to make the calculations tractable.

Means and between-subject standard deviations for subject characteristics and subjective ratings were derived from the raw values of the measures; for all other measures they were derived by back transformation of the log-transformed values. Standard deviations and effects for measures of marker concentrations are shown as factors because of the large between-subject variations (some >100%). Concentrations below the level of detection (22% of the assays for plasma IL-1β, 33% for plasma IL-6, 30% for plasma IL-8, 28% for salivary IL-6, and 22% for salivary IgA) were estimated as approximately half the lowest concentration detected in the respective assays (plasma IL-1β, 2.7 $pg.ml^{-1}$; plasma IL-6, 0.15 $pg.ml^{-1}$; plasma IL-8, 0.58 $pg.ml^{-1}$; salivary IL-6, 0.22 $pg.ml^{-1}$; and salivary IgA, 16 $\mu g.ml^{-1}$).

Errors of measurement were calculated in the spreadsheet[11] as the standard deviation of change scores divided by √2. The most appropriate change scores for this purpose were those between interventions for the morning values. The errors of measurement for the three pairs of interventions were averaged (by averaging their squares). An intraclass (retest) correlation was calculated from errors of measurement using the formula $(SD^2\text{-}error^2)/SD^2$, where SD was the between-subject SD in the morning session for the CC

intervention. For the markers, errors were expressed as factors and correlations were derived from the log-transformed values.

An effect was deemed unclear if its 90% confidence interval overlapped thresholds for substantiveness; that is, if the effect could be substantially positive and negative. An estimate of the smallest substantial change was required to make these inferences: we assumed a standardized change of 0.2 of the between-subject standard deviation in CC am.[13] Clear effects are shown as increases and decreases, with the observed magnitude determined by standardization. The thresholds for moderate and large effects were assumed to be 0.6 and 1.2 of the between-subject standard deviation in CC am.[13]

Results

Marker Concentrations

Marker concentrations are shown graphically in Figure 2. Differences in morning (am) marker concentrations, expressed as ratios with 90% confidence limits and qualitative magnitudes, are shown in Table 2. The differences were small, trivial, or unclear, with the exception of the moderate increase in salivary IgA with CP relative to WP, and the moderate reduction in salivary cortisol with CC relative to CP and WP.

The biggest difference between water and carbohydrate in post-exercise (pm) marker concentrations was a moderate reduction in salivary cortisol following consumption of water (ratio 0.26; 90% confidence limits ×/÷1.5). Other differences were trivial (plasma creatine kinase, plasma IL-1β, plasma IL-6) or unclear (protein carbonyls, salivary IL-6, plasma IL-8, salivary IL-8 and salivary IgA), although it is apparent from Figure 2 that all the unclear differences were trivial.

Correlations between plasma and salivary concentrations for IL-6 and for IL-8 ranged from -0.05 to 0.32; all 90% confidence limits were ~±0.6.

Subjective Ratings

Subjective ratings of coping with exercise, leg soreness, and arm soreness are presented in Figure 3. Differences in mean morning (am) ratings for the three interventions are shown in Table 3. The differences were unclear, with the exception of the reduction in arm soreness with CC relative to WP.

The difference between water and carbohydrate in mean ratings with exercise (pm) was unclear for arm soreness, trivial for leg soreness and moderate for coping with exercise: the swimmers coped better with exercise when water was consumed (difference 8.4; confidence limits ±4.9).

Table 2 Comparison of effects of three dietary interventions (CC, CP and WP) on recovery of marker concentrations. Data are mean ratios for morning (am) sessions with ×/÷90% confidence limits and qualitative magnitudes.

	CP/CC	WP/CC	WP/CP
Plasma creatine kinase	0.83; ×/÷1.4 small	1.1; ×/÷1.4 unclear	1.3; ×/÷1.3 small
Protein carbonyls	0.97; ×/÷1.2 unclear	0.91; ×/÷1.3 unclear	0.94; ×/÷1.3 unclear
Salivary cortisol	2.7; ×/÷2.5 moderate	2.9; ×/÷2.1 moderate	1.0; ×/÷2.4 unclear
Plasma IL-1β	0.81; ×/÷1.3 trivial	0.83; ×/÷1.6 trivial	1.0; ×/÷1.3 trivial
Plasma IL-6	0.44; ×/÷2.4 small	0.57; ×/÷1.7 trivial	1.3; ×/÷1.6 trivial
Plasma IL-8	0.57; ×/÷2.8 small	0.57; ×/÷2.5 small	1.0; ×/÷1.2 trivial
Salivary IL-6	0.88; ×/÷1.9 unclear	1.2; ×/÷1.4 trivial	1.3; ×/÷2.0 unclear
Salivary IL-8	1.3; ×/÷1.3 small	1.0; ×/÷1.6 unclear	0.81; ×/÷1.5 unclear
Salivary IgA	2.1; ×/÷2.3 small	0.61; ×/÷1.7 small	0.30; ×/÷2.2 moderate

CC, carbohydrate beverage during and immediately after exercise; CP, carbohydrate beverage during exercise and carbohydrate-protein mixture immediately after exercise; WP, non-caloric flavored water placebo during exercise and carbohydrate-protein mixture immediately after exercise.

Qualitative outcomes are the magnitudes of clear differences; *unclear* indicates confidence limits were substantial and opposite in sign.

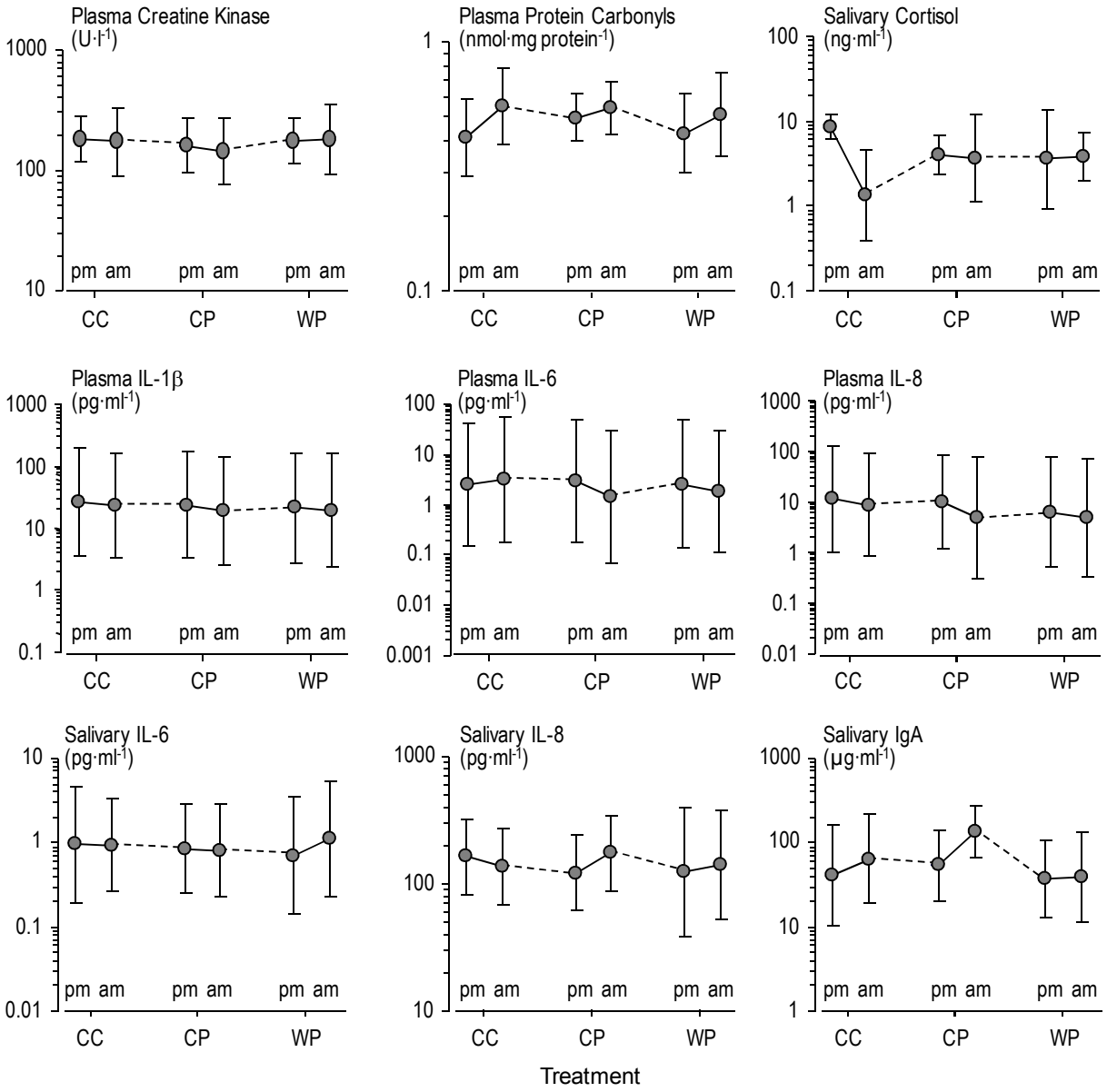


Figure 2 — Marker concentrations after afternoon exercise (pm) and 12 h later, before morning exercise (am). CC, carbohydrate beverage during and immediately after exercise; CP, carbohydrate beverage during exercise and carbohydrate-protein mixture immediately after exercise; WP, non-caloric flavored water placebo during exercise and carbohydrate-protein mixture immediately after exercise. Data are back-transformed means and standard deviations on a log scale.

Table 3 Comparison of effects of three dietary interventions (CC, CP and WP) on recovery of subjective ratings. Data are mean differences for morning (am) session means with ±90% confidence limits and qualitative magnitudes.

	CP - CC	WP - CC	WP - CP
Coping with exercise (0-100)	-2.1; ±5.4 unclear	-2.1; ±8.0 unclear	0.0; ±5.0 unclear
Leg soreness (0-100)	1.5; ±4.9 unclear	3.1; ±7.6 unclear	1.5; ±5.6 unclear
Arm soreness (0-100)	1.9; ±5.8 unclear	4.9; ±6.2 small	3.0; ±6.2 unclear

CC, carbohydrate beverage during and immediately after exercise; CP, carbohydrate beverage during exercise and carbohydrate-protein mixture immediately after exercise; WP, non-caloric flavored water placebo during exercise and carbohydrate-protein mixture immediately after exercise.
Qualitative outcomes are the magnitudes of clear differences; *unclear* indicates confidence limits were substantial and opposite in sign.

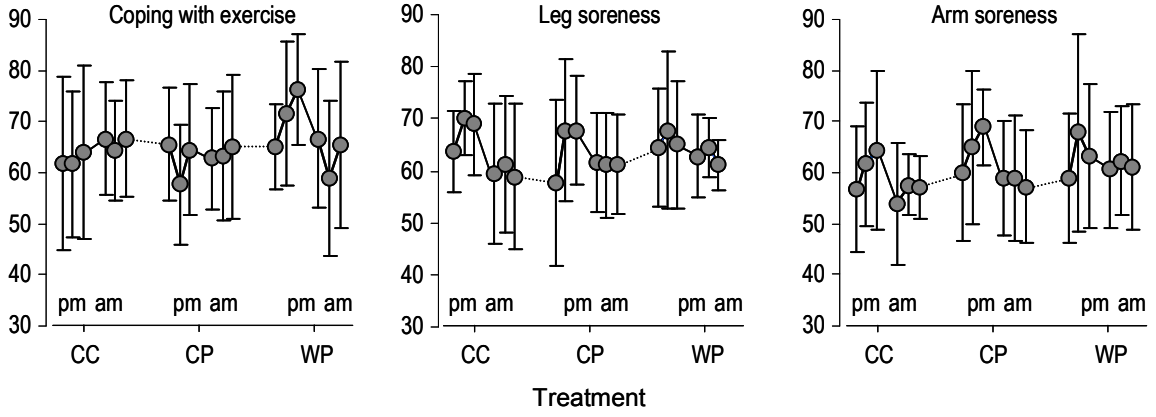


Figure 3 — Ratings of coping with exercise, leg soreness and arm soreness before, during and after afternoon (pm) and morning (am) training sessions with three supplementation interventions. CC, carbohydrate beverage during and immediately after exercise; CP, carbohydrate beverage during exercise and carbohydrate-protein mixture immediately after exercise; WP, non-caloric flavored water placebo during exercise and carbohydrate-protein mixture immediately after exercise. Data are means and standard deviations.

Discussion

This study is an evaluation of a wide range in markers of recovery from exercise in elite swimmers who were subjected to acute dietary interventions during and immediately

after exercise. We did not investigate the effect of the treatments on functional recovery (performance), because the coaches were unwilling to include standardized performance tests. We have provided evidence that the CP intervention induced better recovery in plasma creatine kinase and salivary IgA, but whether these effects indicate substantial clinical changes in muscle damage and mucosal immunity is uncertain: for all treatments, the mean values of creatine kinase concentrations were within normal ranges for athletes[14] and the mean values of salivary IgA concentrations were already high.[15] We have also demonstrated that the inclusion of protein reduces inflammatory responses: CP and WP suppressed plasma cytokines IL-6 and possibly IL-8 in comparison with CC. Further research will be necessary to determine whether these changes are physiologically substantial. Potential recovery impairments with WP are the reduced IgA concentrations and relative to CC also the reduced recovery from arm soreness. The observed differences between the interventions on protein carbonyls were trivial, although effects were unclear.

The CC intervention suppressed morning cortisol relative to the other interventions, but whether a suppression of cortisol would be beneficial is uncertain: the high values that usually occur soon after waking[16] may have beneficial roles in adaptation, stress and energy mobilization.[17,18] Moreover, mean morning values of salivary cortisol were low for all treatments in comparison with the reference range of Laudat et al.[19]

Although the focus of this study was on markers of recovery from training, we also assessed the differences between carbohydrate and water ingestion during exercise on mean subjective ratings with exercise (pm) and on marker concentrations in samples taken immediately after exercise (pm). Differences between carbohydrate and water were trivial or unclear, with two surprising exceptions, both of which are in contrast with previous findings on cyclists and runners[20,21]: post-exercise cortisol was higher with carbohydrate, and the swimmers coped less well with carbohydrate. More research is needed to confirm these effects. The increase in cortisol and the impairment in coping with exercise by consuming carbohydrate during exercise may indeed be the result of the exercise mode (swimming). Another possible explanation is that the swimmers had not fully accustomed to the carbohydrate provided during exercise: the swimmers ingested

little carbohydrate during exercise before the study began, so gastric distress[22] or psychological distress[23] may have influenced cortisol and coping with exercise.

The poor correlations between plasma and salivary measurements for IL-6 and IL-8 show that cytokine concentrations in saliva and plasma samples are not interchangeable, as others have demonstrated.[24] Because of the uncertainty in the correlations, we cannot exclude the possibility of stronger relationships, but we can be confident that the correlations are not high enough to estimate plasma cytokine concentrations via salivary concentrations in individuals.

Conclusion

We have demonstrated clear changes in markers of recovery from exercise in elite swimmers, but the clinical significance of these changes is unclear. Further investigations focused on effects of dietary treatments on functional recovery are needed to meticulously advise athletes and coaches of best-practice.

Acknowledgements

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CHAPTER 2

Acute Effects of a Carbohydrate-Protein Sports Drink on Performance in Swimmers

Abstract

Carbohydrate-protein supplements enhance performance in long-duration exercise, but little is known about the effects on high-intensity short-duration swim performance. ***Purpose:*** To quantify the acute effects of carbohydrate-protein-electrolyte consumption on performance in swimmers. ***Methods:*** In a double-blind randomized crossover with a 1-wk washout, 13 highly trained swimmers (6 females and 7 males; age 17-23 y) consumed either carbohydrate-protein-electrolyte or non-caloric flavored water before and during an incremental 7x200-m step test, and 10 min before a 100-m time trial 24 h later. Diet and training were standardized. Earlobe blood samples were taken to measure lactate and glucose concentrations. We assessed changes in lactate-profile performance, in performance in the last step of the step test, and in performance in the 100-m time trial. We used magnitude-based clinical inferences for effects on performance and mechanistic inferences for effects on lactate and glucose concentrations. ***Results:*** The effects of the supplement on performance time in the last step of the step test and in the time trial were possibly harmful and unlikely to be beneficial: last step of the step test, 0.9% (90% confidence limits ±1.1%); and 100-m time trial, 0.1% (±0.6%). The change in performance in lactate-profile time was unclear. The supplement increased blood glucose moderately in the step test, by 7.4% (±4.0%). ***Conclusion:*** Given the potentially harmful effects of the carbohydrate-protein-electrolyte supplement on performance, we advise against its use in high-intensity short-duration swim performance tests and races.

Key Words*:* athlete, lactate threshold, nutrition, test, time trial

Introduction

The performance benefits of consuming carbohydrate solutions during long-duration exercise (>1 h) have been clearly established in several studies.[1,2] The most popular rationale underlying the advantage of carbohydrate ingestion during prolonged exercise is that, in preventing hypoglycemia and providing a fuel source that is immediately usable by the working muscles, the onset of fatigue might be delayed.[3] This rationale may also explain performance benefits in high-intensity resistance exercise,[4] in high-intensity intermittent exercise[5,6] and in short-duration exercise preceded by a substantial preload.[7-16] The effects of carbohydrate ingestion on performance in short-duration high-intensity exercise that is not preceded by a substantial preload are less clear. Palmer et al.[17] and Jeukendrup et al.[18] have demonstrated that carbohydrate ingestion does not enhance performance significantly in 20-km (~28 min) and 16-km (~26 min) cycling time trials respectively, indicating that short-duration cycling exercise may not depend on carbohydrate availability. To our knowledge, no researchers have quantified acute effects of carbohydrate supplements on exercise of duration less than 10 min that was not preceded by a substantial preload. Furthermore, we have found few publications on the performance effects of acute carbohydrate supplementation in swim exercise.[19,20] In a recent conference abstract,[21] it was suggested that the ingestion of a carbohydrate-protein gel improved performance compared with non-caloric placebo in a high-intensity swim set consisting of 24 200-yd repeats in collegiate swimmers. This finding was interesting, more so because the inclusion of protein in a carbohydrate-supplement had been suggested to be better practice for performance and recovery from exercise compared with carbohydrate-only supplementation in a number of studies.[22-24]

We have observed that swimmers use carbohydrate and carbohydrate-protein supplements before races and in short-duration high-intensity swim sets. As research on effects of this practice in high-intensity short-duration exercise is lacking, we need some evidence that this practice does not impair performance. The purpose of the present study was therefore to evaluate the effects of a carbohydrate-protein-electrolyte supplement vs non-caloric placebo on performance in short-duration swim tests. We chose the widely used incremental 7x200-m step test, as well as a 100-m time trial to mimic competition.

We did not include a carbohydrate-only treatment, because the training program, and therefore the research design, limited us to compare the effects of two treatments only. By measuring blood-lactate and blood-glucose concentrations, we hoped to elucidate some of the potential mechanisms for any observed differences in performance. Although researchers have observed changes in lactate and glucose concentrations with carbohydrate consumption during exercise,[25] there have been no publications on the effects of these supplements on the lactate-threshold outcomes in swim step tests.

Methods

Subject Characteristics

Subjects (6 females and 7 males; age 17-23 y) were highly trained swimmers competing at national (n=5) and/or international (n=8) level and specializing in freestyle (n=6), backstroke (n=5), butterfly (n=1) and breaststroke (n=1). Subject characteristics are shown in Table 1. The swimmers were performing a similar training program consisting of two 2-h swim sessions each day, except for Wednesday and Saturday (morning session only), and Sunday (no session). Sessions started at 6:30 am and 4 pm. Testing was performed in a period of medium-volume training (60-70 km per week). Training and warm-up prior to each test were standardized; the swimmers did not perform dry-land training for at least 82 h before each intervention. All subjects gave written informed consent as required by the AUT University Ethics Committee, which approved this study.

Diet Control

The morning before each intervention, we provided the athletes with breakfast, and a pre-prepared food package for lunch and afternoon snack. The swimmers were instructed to consume only the provided foods and water. Lunch and snack were consumed 4 h and 1.5 h respectively before the start of the session. Meals were similar on all testing days.

Study Design and Supplement Details

In a double-blind randomized crossover with a 1-wk washout, the swimmers consumed either a carbohydrate-protein-electrolyte supplement (Accelerade, PacificHealth Laboratories, New Jersey) or non-caloric flavored water (Thriftee,

Hansells, New Zealand) before and during an incremental 7x200-m step test, and 10 min before a 100-m time trial 24 h later. The carbohydrate-protein drink and the placebo matched colour, taste and texture. The study design is shown in Figure 1. Effects of circadian rhythm were avoided, as testing was performed at the same time of day. The order of treatment was randomized to balance gender and distance of main competitive event.

Table 1 Subject characteristics

	Females (n=6)	**Males (n=7)**
Age (y)	19 ± 1	19 ± 2
Height (cm)	174 ± 5	183 ± 4
Body mass (kg)	67 ± 4	76 ± 6
Recent performance (%)[a]	93 ± 2	92 ± 2
Lactate-profile 200-m time (s)[b]	148 ± 10	135 ± 5
Lactate concentration ($mmol \cdot L^{-1}$)[c]	3.3 ± 0.8	5.2 ± 1.2
Last step of 200-m step test (s)[b]	138 ± 11	125 ± 6
100-m time-trial time (s)[b]	64.6 ± 6.1	57.2 ± 3.8

Data are means ± SD.
[a]Best competitive performance in the main competitive event in the previous year as a percent of world record.
[b]For placebo treatment.
[c]Mean value at which each athlete's lactate-profile time was evaluated.

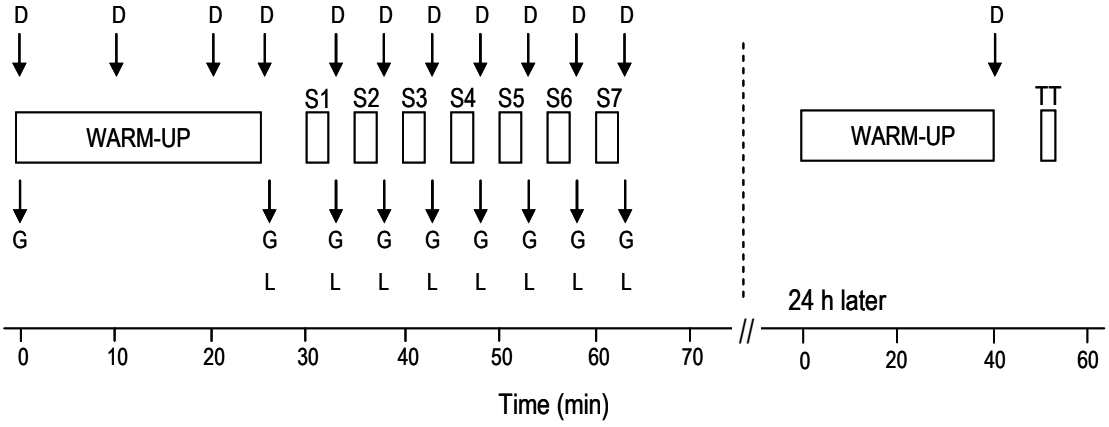

Figure 1 — Study time-line. The order of supplement and placebo was randomized. D, drink (supplement or placebo); S, step of step test; G, glucose measurement; L, lactate measurement; TT, time trial.

Carbohydrate-protein was a combination of sucrose (~80% of total carbohydrate content), trehalose (~10%), maltodextrin (~5%), fructose (~5%) and whey protein concentrate in a ratio of 4:1 carbohydrate:protein. The carbohydrate-protein powder was mixed with water to make a 7% carbohydrate drink. Other supplement details are shown in Table 2. The swimmers consumed 11 equal boluses of the supplement or placebo in the step-test session at times shown in Figure 1. Subjects were asked to verbally report gastrointestinal distress.

Table 2 Supplementation details

	Accelerade	Placebo
Composition		
Carbohydrate (%)	**7.0**	**-**
Protein (%)	**1.8**	**-**
Sodium ($mmol \cdot L^{-1}$)	**30**	**4**
Potassium ($mmol \cdot L^{-1}$)	**6**	**-**
Consumption in step test		
Carbohydrate consumption ($g \cdot kg^{-1}$)	**1.0**	**-**
Mean fluid consumption (L)	**1.0**	**1.0**
Consumption in time trial		
Carbohydrate consumption ($g \cdot kg^{-1}$)	**0.13**	**-**
Mean fluid consumption (L)	**0.13**	**0.13**

Performance Tests

The step test consisted of seven steady-paced 200-m swims on a 5min cycle, graded from easy to maximal. Individualized target times based on each swimmer's personal best time (PB) were calculated prior to testing with the following formula: Step 1, 200-m PB + 35 s; Step 2, 200-m PB + 30 s; Step 3, 200-m PB + 25 s; Step 4, 200-m PB + 20 s; Step 5, 200-m PB + 15 s; Step 6, 200-m PB + 10 s; Step 7, maximal effort swim. The step test was conducted in a 25m pool; all swims started with a push from the wall. The swimmers completed the test using the stroke of their main competitive event, with the exception of butterfly swimmers, who performed the test in freestyle. The 100-m time trial was performed in a 50-m pool; the athletes used a dive start and swam the stroke of their main competitive event. We filmed performance in the time trial and determined times by analyzing the footages with video-analysis software (SiliconCoach, Dunedin, NZ). The 1-

wk standard errors of measurement derived from supplement and placebo trials were: Step 7, 1.6%; lactate-profile time, 1.9%; 100-m time trial, 0.8%.

Blood Sampling

During each step-test session, we collected 100-µl blood samples from the earlobe to assess lactate and glucose concentrations at times shown in Figure 1. Lactate and glucose concentrations were determined using portable hand-held blood-lactate analyzers (Lactate Pro, Arkray, Japan) and blood-glucose analyzers (Ascensia Contour, Bayer, Indiana). All participants were familiar with this regular monitoring procedure. The 1-wk standard errors of measurement derived from supplement and placebo trials ranged from 18% to 39% and from 6% to 13% respectively for lactate and glucose measures.

Statistical Analysis

Analyses were performed using an Excel spreadsheet for crossovers.[26] Means and between-subject standard deviations for subject characteristics were derived from the raw values of the measures; for all other measures they were derived by back-transformation of the log-transformed values. To derive a measure of performance from the lactate concentrations in each step test, we assumed a log-log relationship between lactate concentration and performance time.[27] We used the Forecast function in Microsoft Excel to fit straight lines to lactate plots, and we predicted performance time corresponding to the mean lactate concentration of all samples for the given athlete on the linear part of the athlete's plot.[28] We refer to this measure of performance as lactate-profile time, and it is analogous to a lactate threshold. Errors of measurement were calculated in the spreadsheet as the standard deviation of change scores divided by √2. The effects of the supplement were quantified by calculating mean changes in variables between supplement and placebo.

To make inferences about true (population) values of the effects of the supplement, the uncertainty in the effect was expressed as 90% confidence limits and as likelihoods that the true value of the effect represented substantial change.[29] An estimate of the smallest substantial change was required to make these inferences. For the performance measures represented as lactate-profile and 100-m time-trial time, we based the estimate on variability in performance of top athletes between competitions and assumed a smallest substantial change of 0.25%.[30,31] For the performance measure represented by Step 7, we

assumed a smallest worthwhile effect of 0.5%, because the pre-load represented by the earlier steps appeared to magnify performance error (and presumably also performance effects) by a factor of 2. For lactate and glucose concentrations, we assumed a standardized change of 0.2 of the between-subject standard deviation of resting or warm-up values; the thresholds for moderate and large effects were assumed to be 0.6 and 1.2 of the between-subject standard deviations respectively.[29]

Magnitude-based inferences were clinical for performance measures and mechanistic for lactate and glucose concentrations.[32] With clinical inferences, an effect with possible benefit (>25% chance) was clear if harm was very unlikely (odds ratio of benefit/harm >66) and unclear otherwise; other effects were clearly not beneficial and were reported with their possibility of harm and/or benefit. With mechanistic inferences, an effect was deemed unclear if its 90% confidence interval overlapped thresholds for substantiveness (that is, if the effect could be substantially positive and negative); other effects were clear.

Results

Performance

Mean performance times with the placebo treatment are shown in Table 1. Consumption of carbohydrate-protein-electrolyte possibly impaired performance in Step 7 (mean change in time 0.9%; 90% confidence limits ±1.1%; clinical inferences possible harm, very unlikely benefit) and in the 100-m time trial (0.1%; ±0.6%; possible harm, unlikely benefit). The effect of the supplement on performance expressed as lactate-profile time was unclear (0.2%; ±1.3%).

Lactate and Glucose Concentrations

Figure 2 shows the glucose and lactate concentrations in the step test. Individual concentrations of blood glucose and blood lactate (mmol·L) ranged from 3.4 to 9.3 and 0.7 to 16.2 respectively with the Accelerade treatment, and from 4.2 to 9.3 and 0.7 to 15.2 respectively with the placebo treatment. The glucose concentration (mean ± SD) at the start of the step-test session (before warm-up) was 5.3 ± 0.6 in both the supplement and placebo condition. The differences in glucose concentrations between the supplement and placebo were unclear or small, with the exception of moderate increases in concentrations

immediately after the warm-up (mean change 6.9%; 90% confidence limits ±4.5%), Step 6 (11%; ±7.3%), and Step 7 (10%; ±8.7%). The difference between supplement and placebo in the mean of the glucose concentrations in samples after warm-up and each step of the step test was moderate (7.4%; ±4.0%). The differences in lactate concentrations between supplement and placebo were all unclear, although it is apparent from Figure 2 that the observed differences were trivial.

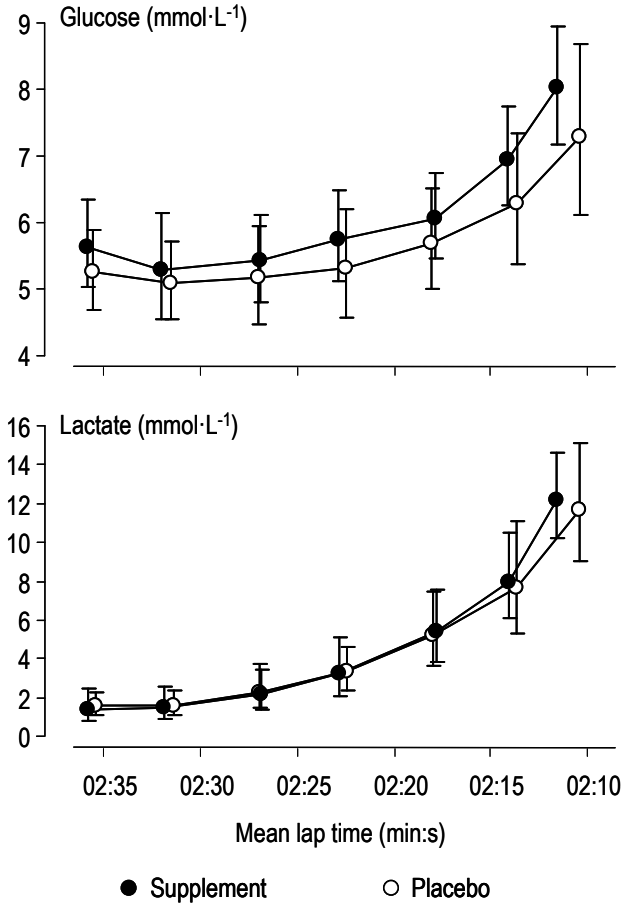

Figure 2 — Concentrations of glucose and lactate in the step test. Data are back-transformed means and standard deviations.

Discussion

To our knowledge, this study is the first to investigate the acute effects of any carbohydrate supplement on performance in high-intensity short-duration swim exercise that was not preceded by a substantial preload or overnight fast. In spite of the relatively small sample size, the effects of the supplement on performance in the last step of the step test and in the time trial were clear, owing to high reliability in these measures: the performance measures of the step test were as reliable as the best of these tests[33] and the 100-m time trial was as reliable as competition performance in top swimmers.[31]

Blood-glucose concentrations did not appear to be low at any sampling time for any swimmer, and they increased with exercise in both treatments. The increase in blood glucose with the placebo treatment was probably the result of an increase in glucose output from the liver.[34] The higher blood-glucose values with carbohydrate-protein administration were presumably the result of glucose absorption through the small intestine. We did not observe any hypoglycemia, which can occur with carbohydrate ingestion,[17] either because carbohydrate was ingested during exercise,[35] or because any hypoglycemia had resolved after the 25min warm-up. The supplement impaired performance in the last step of the 7x200-m step test. A possible explanation for this impairment is gastrointestinal distress. Not measuring gastrointestinal distress on a Likert or similar scale was a limitation of this study. Although the subjects were asked to verbally report gut discomfort, they may have failed to do so. Another possible explanation for the performance impairment in the last step is an increase in blood-glucose utilization and a sparing of muscle glycogen with supplement ingestion.[36] If this change in metabolism increased the demand for ATP, high-intensity exercise could be impaired. More research is needed to investigate these possibilities. Other researchers have shown that the sweet taste of a carbohydrate drink is enough to enhance performance of longer-duration exercise,[37] presumably via the perception of effort or fatigue. This mechanism seems unlikely to explain our findings, unless there are two classes of chemo-receptors for sweetness (carbohydrate and saccharin). In any case, the carbohydrate-protein sports drink *impaired* performance in the last step of the step test.

The possible performance impairment in the 100-m time trial with carbohydrate-protein did not arise simply from sampling variation, it may have been caused by a surge in insulin.[37] Other explanations in terms of carbohydrate availability seem unlikely, because the timing of intake would not have allowed much of the carbohydrate to be absorbed, and the duration of exercise would have been too short.[17] The fact that performance impairment was possible in the time trial may also indicate that the use of the supplement during the hard exercise of the step test the day before had little effect on functional recovery.

Practical Applications

We advise against the use of a carbohydrate-protein supplement before or during short-duration high-intensity swim tests and competition. In the absence of more evidence, best practice still remains that swimmers use these supplements immediately after exercise for better recovery.

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CHAPTER 3

Monitoring Acute Effects on Athletic Performance with Mixed Linear Modeling

Abstract

There is a need for a sophisticated approach to track athletic performance and to quantify factors affecting it in practical settings. **Purpose:** To demonstrate the application of mixed linear modeling for monitoring athletic performance. **Methods:** Elite sprint and middle-distance swimmers (3 females, 6 males; age 21-26 y) performed 6-13 time trials in training and competition in the 9 wk prior to and including Olympic-qualifying trials, all in their specialty event. We included a double-blind, randomized, diet-controlled crossover intervention, in which the swimmers consumed caffeine (5 $mg \cdot kg^{-1}$ body mass) or placebo. The swimmers also knowingly consumed varying doses of caffeine in some time trials. We used mixed linear modeling of log-transformed swim time to quantify effects on performance in training vs competition, in morning vs evening swims, and with use of caffeine. Predictor variables were coded as 0 or 1 to represent absence or presence of each condition and included as fixed effects. Date of each performance test was included as a continuous linear fixed effect and interacted with the random effect for athlete to represent individual differences in linear trends in performance. **Results:** Most effects were clear, owing to high reliability of performance times in training and □ å □ □ □□Ĥ□□ □Ā□Ĥ□□ □□□ □□□□ □□□□□Ĥ□□ □□□□ÿ□□□□□□И□□□□□□□□□□ .8% per 4 wk. The swimmers performed substantially better in evenings vs mornings and in competition vs training. A 100-mg dose of caffeine enhanced performance in training and competition by ~1.3%. There were substantial but unclear individual responses to training and caffeine (SD of 0.3% and 0.8%, respectively). **Conclusion:** Mixed linear modeling can be applied successfully to monitor factors affecting performance in a squad of elite athletes.

Key Words: ELITE ATHLETES, CAFFEINE, METHOD, TRAINING

Introduction

The primary aim of sport scientists working with elite athletes is to assess the effects of training and nutritional or other treatments on performance. Several mathematical models have been suggested for analyzing effects of treatments on performance (2,4,11,17,28,29). Repeated measures ANOVA is a commonly used method, but it can lead to loss of power when there are missing values in a series of repeated measurements: either the entire trial with a missing value has to be deleted, or the entire series of values of each subject with a missing value has to be deleted. A better approach is mixed modeling, which overcomes the missing-value problem and in addition allows specification and estimation of different sources of variation or error (30). For example, in tracking performance using training and competition time trials, performance could be more variable in training.

In this article, we report an analysis with mixed modeling in which we have devised a novel coding method to account for various factors that could affect performance. We monitored performance in a squad of elite swimmers preparing for Olympic-qualifying trials and assessed changes in performance arising from training vs competition, morning vs evening swims, and with use of caffeine or placebo.

Methods

Subject characteristics. Nine highly trained swimmers (age range, 21-26 y) competing at international level and specializing in 400m freestyle (n=1), 100m backstroke (n=1), 200m backstroke (n=2), 100m butterfly (n=1), 200m butterfly (n=2), 100m breaststroke (n=1) or 400m individual medley (n=1) took part in this study. Subject characteristics are shown in Table 1. The swimmers were performing a similar training program consisting of two 2-h swim sessions each day, except for Wednesday and Saturday (morning session only), and Sunday (no session). Sessions started at 6:30 am and 4 pm. All subjects gave written informed consent as required by the AUT University Ethics Committee, which approved this study.

Study design. The swimmers performed 2-8 time trials in training and 2-7 in competition in the 9 wk prior to and including Olympic-qualifying trials; 0-4 time trials

were performed in the morning and 4-10 were performed in the evening. Morning time trials were performed between 9 and 11:30 am, and evening time trials were performed between 5 and 8 pm. All trials were performed in the stroke and distance of each swimmer's main event, with a standardized individualized competition warm-up, leading-in music and cheering from fellow athletes. The swimmers used a race swim-suit of the same size and brand in all trials. The warm-up consisted of stretching, 1-2 km of swimming and mental preparation. We monitored performance times and use of caffeine in these time trials. All trials were performed in a 50-m pool with a water temperature of 27°C and an ambient temperature of 24°C. Data for one elite swimmer are shown in Figure 1.

TABLE 1. Characteristics of the squad of elite swimmers.

	Females (n=3)	**Males (n=6)**
Age (y)	24 ± 2	23 ± 2
Height (cm)	175 ± 5	184 ± 3
Body mass (kg)	66 ± 4	80 ± 5
Recent performance (%)[a]	97.3 ± 0.2	96.5 ± 2.3

Data are means ± SD.

[a]Best competitive performance in the main competitive event in the previous year as a percent of world record.

The athletes consumed caffeine of varying doses in some time trials and a fixed dose (5 $mg \cdot kg^{-1}$ body mass) or placebo in a double-blind, diet-controlled crossover manner in two training time trials ≤2 wk apart. The order of treatment in the crossover was randomized to balance gender and distance of main competitive event. The caffeine was consumed in tablet-form except for the crossover intervention, in which the swimmers ingested capsules containing either placebo (custard powder, Hansells, NZ) or crushed 100-mg caffeine tablets (No-Doz, Key Pharmaceuticals, Australia). These capsules were ingested with lemon-flavoured water, and were consumed blindfolded, even though the custard powder and caffeine had a similar colour and texture. The caffeine and placebo were always consumed 75 min before the time trial.

Diet. We standardized diet only for the two time trials in the caffeine crossover intervention. All subjects completed a 36-h diet-diary before the first time trial of this intervention and repeated this diet for the second time trial. The athletes were also

instructed to refrain from caffeine and alcohol for three days before these two time trials. Only one swimmer consumed caffeine on a regular basis.

Focus, sleep quality and perception of having consumed caffeine. The athletes completed a questionnaire the morning after each time trial part of the caffeine crossover intervention. They were asked to rate pre-time-trial focus and sleep quality between 0 and 100 on a 21-point scale anchored throughout with descriptors ("0, no focus at all" through "100, extremely focused" and "0, no sleep" through "100, perfect sleep" respectively). This scale was adapted from Ritchie and Hopkins (26). The questionnaire was also used to record the time that the athletes went to bed, the time required to fall asleep, the duration of sleep, and the number of times of waking up. The swimmers were also asked to rate their perception of having consumed caffeine or placebo as follows: "almost certainly not caffeine", "probably not caffeine", "unsure", "probably caffeine", or "almost certainly caffeine"; we coded this variable from 0 to 4 respectively. We also assigned this variable a value of 0 for all swims where no caffeine was consumed knowingly and a value of 4 for swims when caffeine was consumed knowingly. We then calculated the placebo effect as the difference between the effect on performance of a perception of 4 and 0 (all other effects held constant).

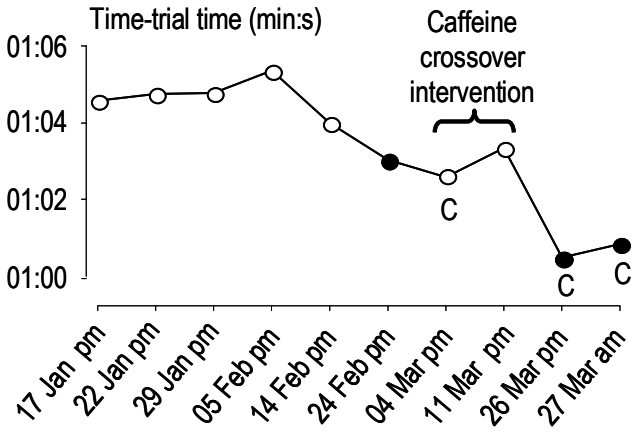

Figure 1—Time trials in 100-m breaststroke performed in training (○) and competition (●) in one elite swimmer. C indicates use of caffeine in the corresponding time trial.

Analysis. We used the mixed model procedure (Proc Mixed) in the Statistical Analysis System (Version 9.2, SAS Institute, Cary, NC) to quantify the changes in performance that occurred in training vs competition, in morning vs evening swims, and with use of caffeine and placebo. Predictor variables were coded as 0 or 1 to represent absence or presence of a condition and included in the model as fixed effects. Caffeine dose was included in the model as the number of capsules ingested. We did not include an interaction between caffeine dose and gender, because a meta-analysis of the effects of caffeine on performance showed a trivial effect of gender (WGH, unpublished observations). Date of each performance test and caffeine (present or absent) were included as numeric linear fixed effects to estimate their mean effects. We also interacted these variables with the random effect for athlete to estimate individual differences in their effects. One residual variance was specified for the training time trials and another for the competition time trials, and the square roots of these variances were interpreted as the typical errors of the performance test to interpret the reliability of the test under the conditions of the study. We allowed for negative variance to estimate these individual differences, because treatment effects, training effects and sampling variation could reduce variability in performance. Confidence limits produced by the mixed-model procedure for the standard deviations representing individual differences are only approximate with our small sample size. A plot of residuals from the analysis vs date was examined to assess the appropriateness of the use of a linear effect for date.

To investigate the effect of caffeine on longer vs shorter swims, duration of the time trial was included in the model as a log-transformed value interacted with caffeine (present or absent) and number of caffeine capsules. To avoid a problem with collinearity, the duration chosen was that for a swim that would merit 900 points on the Point Scoring of the Fédération Internationale de Natation. The effect of time trial duration on the effect of caffeine dose was estimated as the change in the effect of caffeine for a doubling of time-trial time. We opted for a factor effect of time-trial duration rather than a linear effect, because a linear effect would imply the same change in the effect of caffeine on performance per minute of exercise, whether the exercise lasted 5 min or 1 h. A doubling of time-trial time implies the same change in the effect of

caffeine with an increase in time-trial duration from 5 to 10 min as for 1 h to 2 h, which is more realistic.

We used an Excel spreadsheet for crossovers (19) to determine the changes in measures derived from the focus and sleep questionnaire. For these variables, means and between-subject standard deviations were derived from the raw values of the measures; and errors of measurement were calculated in the spreadsheet as the standard deviation of change scores divided by √2. An intraclass (retest) correlation was calculated from errors of measurement using the formula $(SD^2\text{-error}^2)/SD^2$. For reliability of measurement in the performance test, we calculated the typical error expressed as a percentage of the subject's mean score.

To make inferences about true (population) values of an effect, the uncertainty in the effect was expressed as 90% confidence limits and as likelihoods that the true value of the effect represents substantial change (harm or benefit) (5). An estimate of the smallest substantial change in a given dependent variable was required to make these inferences. The threshold change in performance for benefit and harm was established as 0.24% (0.3xwithin-athlete race-to-race variability in performance of 0.8%; thresholds for moderate and large effects were 0.72 and 1.3% respectively (20). For the dependent variables derived from the questionnaire, we assumed that the smallest substantial change was a standardized change of 0.2 of the between-subject standard deviation of all (placebo and caffeine) values; the thresholds for moderate and large effects were assumed to be 0.6 and 1.2 of the between-subject standard deviations respectively (20).

Magnitude-based inferences were categorized as clinical for performance measures and mechanistic for other measures (20). With clinical inferences, an effect with possible benefit (>25% chance) was clear if harm was very unlikely (odds ratio of benefit/harm >66) and unclear otherwise; other effects were clearly not beneficial. With mechanistic inferences, an effect was deemed unclear if its 90% confidence interval overlapped thresholds for substantiveness (that is, if the effect could be substantially positive and negative); other effects were clear.

Results

Performance times were highly reliable in training and competition (typical errors 0.9 and 0.8% respectively). The plot of residuals vs date of the performance test showed a uniform scatter that justified the use of a linear effect of date on performance. Performance time improved by 0.8% (qualitative outcome moderate; 90% confidence interval 0.4% to 1.2%) per 4 wk of training, with individual differences (standard deviation) in the trend of 0.3% (unclear; -0.6 to 0.4%) per 4 wk. The swimmers performed better in evenings vs mornings by 0.6% (small; 0.1 to 1.0%) and in competition vs training by 1.4% (large; 0.9 to 1.9%).

A 100-mg dose of caffeine enhanced performance in 1min training and competition time trials by 1.3% (large; -0.3 to 2.8% and 0.1 to 2.6%, respectively); each additional 100 mg reduced the benefit slightly by 0.1% (unclear; -0.5 to 0.3%). Only one swimmer (the habitual caffeine user) experienced an increase in swim time with caffeine in the crossover intervention, which is consistent with observed small individual differences (standard deviation) in the effect of caffeine of 0.8% (unclear; -1.4 to 0.7%). The effect of a doubling of time-trial time on the effect of caffeine was -0.3% (unclear; -1.5 to 0.9%). The placebo effect was a slight improvement of 0.2% (unclear; -1.0 to 1.4%).

In the double-blind cross-over intervention, most swimmers were able to identify consumption of caffeine: four swimmers were almost certain they had consumed caffeine, four swimmers perceived that they probably had consumed caffeine, and the one athlete that was a regular user of caffeine was almost certain of not having consumed caffeine. Reliability in measures derived from the questionnaire expressed as error of measurement (and retest correlation) were: focus (0-100 scale), ±6.7 (0.53); bedtime, ±67 min (-0.03); sleep quality (0-100 scale), ±11 (0.52); duration of sleep ±63 min (0.16); time required to fall asleep, ±17 min (0.49); and number of times of waking-up, ±0.77 (0.47). Despite this poor reliability of measurement, we were able to identify some small effects with caffeine intake: an increase in focus (change in mean 4.4; 90% confidence limits ±8.5), a decrease in duration of sleep (-47; ±56), and an increase in time required to fall asleep (16; ±15). Other measures were unclear.

Discussion

We successfully used mixed linear modeling of elite swimmers' performance times to demonstrate and quantify individual trends in performance with training, better performance in evenings vs mornings, better performance in competition vs training time trials, and better performance with caffeine. We were able to quantify these changes in performance in spite of the small sample size, because some of the effects were large and because there were multiple observations in each subject. The high reliability in the time trials used to monitor performance was another important reason: test-retest reliability matched measures in previously published findings on reliability in competition performance in top athletes (25).

We modeled a linear effect for the date of the test because the data showed linear rather than nonlinear trends. This linear effect provided the coach with a measure of change in performance with training that was valuable to assess the training program overall and to identify individual responses with training. We have not attempted to identify which of the many outcomes of training (fitness, pacing, fatigue, technique, psychological state, etc.) were responsible for the improvement in performance. Wearing a new race swim-suit may have contributed to the performance increase in competition time trials. Tapering undoubtedly contributed to the substantial improvement in performance in competition time trials performed in the last week (7).

The performance increase with swimming in the evening vs the morning was substantial but smaller in magnitude than that in previous research (for reviews see 3,15). Baxter and Reilly (6) found improvements of 1.9% and 0.8% in 100-m and 400m swim time respectively when the swims were performed at 5 pm vs 9 am. Deschodt et al. (13) reported a 3.6% improvement in swim time in 50-m time trials performed at 6 pm vs 8 am. The diurnal effect on swim performance may have been smaller in the present study either because of the extensive warm-up (13), or because of the nature of the performance tests (competitions and competition simulations), or because the morning time trials were performed between 9 and 11:30 am. Indeed, Kline et al. (22) observed little change in performance in 200-m swim time trials performed at 5 pm vs 11 am. In any case, it would be sensible to include strategies for using this change in performance to the athletes' advantage; for example, training might be more effective in the afternoon, and using

strategies for resynchronizing the circadian rhythm after transmeridional travel might be worth considering (15,22).

The swimmers performed better in competition than in training, even though the training time trials were competition simulations. It is already well-known that athletes perform better in competition because of higher arousal, competitive stress, less fatigue, and/or tapering for the competition event. The novelty of this project is that we were able to quantify the effect, which may be an aid for the coach to predict competition performance.

We found that caffeine ingested before a swim time trial substantially enhanced performance. Effects of caffeine on endurance performance are well-established, but its effects on short-term endurance and sprinting in well-trained subjects are less clear (1,8,10,12,31). In particular, a recent conference abstract showed little effect of a low dose of caffeine (2 $mg \cdot kg^{-1}$ body mass) on 100-m swim performance in elite athletes (10). One of the reasons for this discrepancy with our findings may be the timing of caffeine intake: the swimmers in the present study ingested the caffeine 75 min before the time trials, whereas in the previous study it was 60 min.

There have been few studies of the dose-response of caffeine on high-intensity, short-term performance in well-trained subjects (1,8). In comparing the effects of doses of 6 and 9 $mg \cdot kg^{-1}$ caffeine in a 2000m rowing time trial lasting ~7 min, Bruce et al. found similar ergogenic effects in competitive oarsmen (8), while Anderson et al. reported a greater effect with the higher dose of caffeine in competitive oarswomen (1). We observed that an intake of caffeine greater than 100 mg did not further improve performance, although this finding was unclear. Pending further research on the effect of dose of caffeine on swimming performance, swimmers should use a dose of ~100 mg, or ~1.3 $mg \cdot kg^{-1}$.

The effect of a doubling of time-trial time on the effect of caffeine was unclear. We observed that the effect of caffeine was slightly decreased when the duration of the time trial was doubled, but we would need more observations to be confident.

Although the measures of focus and sleep were not obtained with a validated questionnaire, 5 mg caffeine$\cdot kg^{-1}$ body mass increased focus before the time trial, which is consistent with previous research (18,27). We also measured the effect of caffeine on

sleep after the time trial, a concern for elite athletes (9). Studies have shown that even low doses of caffeine decrease sleep quality (10,23). The present study confirms that 5 mg caffeine·kg^{-1} body mass consumed ~7 h before bedtime had a harmful effect on sleep. The implications for subsequent competition performance need further investigation. On the basis of our findings, swimmers and presumably other athletes in sports with similar performance times should use the relatively low dose of caffeine (100 mg) that we have found to be effective.

Caffeine improved performance in the crossover intervention in all but one athlete, who consumed caffeine on a regular basis. The effects of caffeine on performance may be attenuated in caffeine-habituated individuals (16). The athletes were instructed not to consume caffeine in the three days before each time trial, so either three days abstinence is not sufficient to resensitize to caffeine, or the athlete failed to comply. A check on compliance would have required testing for metabolites of caffeine in urine samples, which was beyond the scope of this study. Whether three days of abstinence is sufficient to resensitize to caffeine is unclear from published work, although a meta-analysis might provide an answer. It may therefore be worthwhile to investigate how long athletes need to refrain from caffeine for making maximum use of its ergogenic potential. Similarly, studies examining the interaction of caffeine with other proven ergogenic aids, such as carbohydrate (21), creatine (14) and bicarbonate (24) are important, since this scenario is common in athletes.

There was considerable uncertainty in the estimates of individual responses to training and caffeine. Such uncertainty will be a problem in future applications using mixed modeling with a small number of subjects unless it is possible to obtain many more observations for each subject. There are several other issues with the application of mixed modeling to monitor athletic performance. If the error of measurement is uniform between trials within athletes and between subgroups of athletes, it is possible to use the general linear model (or simply multiple linear regression). With either mixed or general linear modeling, the crucial step is the coding of the presence or absence of treatments or other factors affecting performance as predictors with values of 1 or 0. Mixed linear modeling is still required to obtain confidence limits for estimates of standard deviations representing individual responses and individual differences, although, as previously

noted, a considerable number of observations and/or large sample size are needed. Mixed and general linear modeling also allow modeling of trends as polynomials, when data show nonlinear trends. More complex curvature requires nonlinear mixed modeling, which in the Statistical Analysis System is a challenging procedure.

In summary, we successfully used mixed linear modeling of elite swimmers' performance times and a novel coding method to demonstrate and quantify:

- individual trends in performance with training and use of caffeine;
- a substantial performance increase in evenings vs mornings;
- a substantial performance increase in competition vs training time trials;
- a substantial performance increase with caffeine.

We were able to detect and quantify these changes in performance owing to high-reliability of the performance test, multiple observations in the season, and that some effects were large in magnitude.

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CHAPTER 4

Effects of Acute Carbohydrate Supplementation on Endurance Performance: a Meta-Analysis

Abstract

Research on the performance effects of acute carbohydrate supplementation is comprehensive. Here we present the first meta-analytic review of this research. **Methods:** Eighty-eight randomized crossover studies provided 155 estimates for performance effects in time-to-exhaustion tests or in time trials with or without a preload. For the mixed-model meta-analysis, all effects were converted into percent changes in mean power in a non-preloaded time trial and weighted using percent standard errors derived from exact p-values (in a minority of studies) or from estimated errors of measurement (in all other studies). Publication bias was assessed with a plot of t-values for the random-effect solutions vs standard errors. Probabilistic inferences were derived with reference to thresholds for small, moderate and large effects on performance of 0.5, 1.5 and 2.7%. **Results:** Publication bias was reduced by excluding studies with a standard error >1.25%. In the remaining 73 studies and 122 estimates, the meta-analyzed performance effects of carbohydrate supplements ranged from clear large improvements of ~6% to clear moderate impairments of ~2%. The best supplement inferred from the analysis consisted of a ~3-10% carbohydrate-plus-protein drink providing ~0.7 $g \cdot kg^{-1} \cdot h^{-1}$ glucose polymers, ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ fructose and ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ protein. Substantial increases in the benefit of a supplement were probably small with an additional 9-h fast and with inclusion of ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ protein, probably small-moderate with ingesting the first bolus not at the start of exercise but 1-4 h before exercise, and possibly small with increasing the frequency of ingestion by three boluses per hour. Substantial reductions in the benefit of a supplement were possibly moderate with a supplement providing >0.25 $g \cdot kg^{-1} \cdot h^{-1}$ fructose, and possibly small with an increase in ambient temperature of 10ºC. The effect in subjects with VO_2max higher by 10 $ml \cdot min^{-1} \cdot kg^{-1}$ was probably trivial, and the effects of exercise

duration were dependent on the concentration of carbohydrate plus protein in the supplement. The effect of including salt was unexpectedly trivial, and the effect of gender was unclear. **Conclusions:** Carbohydrate supplements with an appropriate composition and administration regime can have large benefits on endurance performance. More research and better reporting are required to investigate the moderating effects of gender and salt.

Introduction

The majority of publications on the performance effects of acute ingestion of carbohydrate and carbohydrate-protein supplements show an ergogenic effect (for reviews see Bosch[1] or Jeukendrup et al.[2]). The underlying mechanism is believed to be a fatigue delay that arises from the maintenance of high rates of carbohydrate oxidation necessary to sustain exercise intensity. In view of the long history of this research, the lack of a published meta-analysis is surprising. Meta-analysts may have been overawed by the diversity in performance tests and the poor reporting of inferential statistics. We have devised ways for converting performance outcomes in time-to-exhaustion tests and time trials to a common metric and for dealing with the lack of inferential information. We have derived meta-analyzed effects of acute carbohydrate supplementation on performance with moderating effects for differences in subject characteristics (gender and level of athletes), supplement characteristics (type, timing and amount of carbohydrate ingestion, and the inclusion of protein and/or electrolytes), exercise protocols (any preload, and the type and duration of exercise), ambient temperature, and fasting time.

Methods

1.1 Study Selection

We used Google Scholar to search for crossover investigations of acute effects of carbohydrate and/or carbohydrate-protein supplements on performance published between 1979 and 2009. Reference lists in reviews and research articles were also examined. Table 1 shows the study-estimate characteristics for studies that were included in the analysis. We considered all studies in which carbohydrate supplements were

consumed with or without protein on the day of a physical performance test, including consumption before and/or during the test. Studies were excluded for the following reasons: published only as conference abstracts; substantial rest-intervals in the preload (e.g., rest:work>1:4); >5 min rest between preload and performance test; performance tests with other than continuous exercise; an inappropriate control (e.g. no fluid consumed in the control); unrealistically high error and performance effect (probably arising from use of a poor ergometer); investigations of supplements with carbohydrates other than glucose, sucrose, fructose or glucose polymers (including maltodextrins); investigations of supplements containing any substances other than carbohydrate, protein and electrolytes, although we included supplementation of high-carbohydrate foods with known approximate content of carbohydrates and protein; a substantial preload preceding a time-to-exhaustion test; glycogen-depleting protocols other than an overnight fast before the start of exercise; and a study of children. A list of these references is available on request.

Table 1. Study-estimate characteristics, shown in chronological order by year and alphabetical order within years.

Reference	Sample size	VO_2 max[a]	Supplement	CHO+ protein (%)[b]	TT or TTE	Exercise protocol[c]	Exercise duration (min)[d]	Power effect (%)[e]	Power SE (%)[f]
Foster 1979[3]	8M,8F	57	Glucose	25	TTE	100%	6	0.4	0.7
Foster 1979[3]	8M,8F	57	Milk[g]	8.3	TTE	100%	6	0.4	0.7
Foster 1979[3]	8M,8F	57	Glucose	25	TTE	80%	53	-2.3	0.7
Foster 1979[3]	8M,8F	57	Milk[g]	8.3	TTE	80%	53	-0.1	0.7
Ivy 1979[4]	7M,2F	60	Polycose	7.5	TT	120 min	120	2.0	1.1
Felig 1982[5]	10M	49	Glucose	5.0	TTE	60-65%	164	0.7	0.9
Felig 1982[5]	9M	49	Glucose	10	TTE	60-65%	148	1.3	1.0
Coyle 1983[6]	9M,1F	59	Glucose polymers	11	TTE	74%	134	1.9	0.6
Bjorkman 1984[7]	8M	56	Glucose	7.0	TTE	68%	116	2.7	0.8
Bjorkman 1984[7]	8M	56	Fructose	7.0	TTE	68%	116	-0.3	1.1
Decombaz 1985[8]	10 M+F	63	Fructose	20	TT	15 min	29	-0.1	1.0
Coyle 1986[9]	7M	70	Glucose polymers	14	TTE	71%	181	3.9	1.0
Gleeson 1986[10]	6M	47	Glucose	18	TTE	73%	96	1.6	0.4

Table 1. Study-estimate characteristics, shown in chronological order by year and alphabetical order within years.

Reference	Sample size	VO_2 max[a]	Supplement	CHO+ protein (%)[b]	TT or TTE	Exercise protocol[c]	Exercise duration (min)[d]	Power effect (%)[e]	Power SE (%)[f]
Flynn 1987[11]	8M	64	CHO mix	5.0	TT	120 min	120	-1.0	1.8
Flynn 1987[11]	8M	64	CHO mix	10	TT	120 min	120	-4.1	1.8
Flynn 1987[11]	8M	64	CHO mix	10	TT	120 min	120	0.4	1.8
Hargreaves 1987[12]	6M	61	Glucose	21.4	TTE	75%	93	0.0	1.2
Hargreaves 1987[12]	6M	61	Fructose	21.4	TTE	75%	93	-0.3	1.2
Murray 1987[13]	13M	45	Glucose polymers	5.0	TT	480 pedal revs	51	-0.2	0.9
Murray 1987[13]	13M	45	CHO mix	6.0	TT	480 pedal revs	65	-0.9	0.9
Murray 1987[13]	13M	45	CHO mix	7.0	TT	480 pedal revs	65	1.4	0.9
Neufer 1987[14]	10M	60	CHO mix	11.3	TT	15 min	45	3.9	1.0
Neufer 1987[14]	10M	60	Sucrose	11.3	TT	15 min	45	4.1	1.0
Sasaki 1987[15]	5M	58	Sucrose	18	TTE	80%	40	5.8	1.3
Davis 1988[16]	7M	63	Glucose	6.0	TT	270 pedal revs	27	-0.2	1.2
Davis 1988[16]	7M	63	Glucose	6.0	TT	270 pedal revs	159	4.1	2.1
Davis 1988[17]	19M	64	CHO mix	6.0	TT	~120 min	129	0.5	0.8
Davis 1988[17]	19M	64	Glucose	2.5	TT	~120 min	129	0.5	0.8
Mitchell 1988[18]	8M	60	CHO mix	5.0	TT	12 min	68	3.4	1.2
Mitchell 1988[18]	8M	60	CHO mix	6.0	TT	12 min	68	2.9	1.2
Mitchell 1988[18]	8M	60	CHO mix	7.5	TT	12 min	68	5.0	1.2
Coggan 1989[19]	6M	65	Exceed	11	TTE	70%	169	2.7	0.7
Maughan 1989[20]	6M	53	Dioralyte	4.0	TTE	70%	76	3.0	1.2
Maughan 1989[20]	6M	53	CHO mix	35.5	TTE	70%	76	0.7	1.2
Maughan 1989[20]	6M	53	Glucose syrup	35.5	TTE	70%	76	0.6	1.2
Maughan 1989[20]	6M	53	Fructose syrup	35.5	TTE	70%	76	-2.4	1.2
Mitchell 1989[21]	10M	63	CHO mix	6.1	TT	15 min	94	2.1	1.2
Mitchell 1989[21]	10M	63	CHO mix	12.2	TT	15 min	94	4.7	1.2
Mitchell 1989[21]	10M	63	CHO mix	18.2	TT	15 min	94	2.8	1.2
Mitchell 1989[21]	10M	63	CHO mix	12.2	TT	15 min	94	3.6	1.2
Murray 1989[22]	7M,5F	48	Sucose	6.0	TT	1200 pedal revs	73	1.2	0.5

Table 1. Study-estimate characteristics, shown in chronological order by year and alphabetical order within years.

Reference	Sample size	VO_2 max[a]	Supplement	CHO+ protein (%)[b]	TT or TTE	Exercise protocol[c]	Exercise duration (min)[d]	Power effect (%)[e]	Power SE (%)[f]
Murray 1989[22]	7M,5F	48	Sucrose	8.0	TT	1200 pedal revs	73	0.6	0.5
Murray 1989[22]	7M,5F	48	Sucrose	10	TT	1200 pedal revs	73	0.1	0.5
Murray 1989[23]	9M,3F	53	Fructose	6.0	TT	600 pedal revs	183	-1.3	0.6
Murray 1989[23]	9M,3F	53	Sucrose	6.0	TT	600 pedal revs	183	0.1	0.6
Murray 1989[23]	9M,3F	53	Fructose	6.0	TT	600 pedal revs	183	-1.5	0.6
Sherman 1989[24]	8M,2F	59	CHO mix	20	TT	~45 min	103	5.4	1.0
Powers 1990[25]	9M+F	63	Exceed	7.0	TTE	85%	36	1.0	1.0
Powers 1990[25]	9M+F	63	Exceed	7.0	TTE	85%	40	-0.2	1.0
Williams 1990[26]	9M	59	CHO mix	5.0	TT	30 km	129	3.6	1.6
Williams 1990[26]	9M	59	CHO mix	5.0	TT	30 km	129	2.7	1.6
Murray 1991[27]	8M,2F	51	Glucose	6.0	TT	4.8 km	95	7.3	2.2
Murray 1991[27]	8M,2F	51	Glucose	12	TT	4.8 km	95	5.2	2.2
Murray 1991[27]	8M,2F	51	Glucose	18	TT	4.8 km	95	7.8	2.2
Sherman 1991[28]	9M	58	Glucose	20	TT	~45 min	127	2.7	1.1
Sherman 1991[28]	9M	58	CHO mix	39.9	TT	~45 min	127	2.6	1.1
Thomas 1991[29]	7M	55	Glucose	17.5	TTE	65-70%	99	1.5	1.1
Thomas 1991[29]	7M	55	Lentils[g]	25	TTE	65-70%	99	3.0	1.1
Thomas 1991[29]	7M	55	Potato[g]	18.5	TTE	65-70%	99	-0.3	1.1
Wright 1991[30]	8M,1F	63	Exceed	5.1	TTE	70%	201	2.1	0.9
Wright 1991[30]	8M,1F	63	Exceed	2.6	TTE	70%	201	3.7	0.9
Wright 1991[30]	8M,1F	63	CHO mix	7.1	TTE	70%	201	4.8	0.9
Millard-Stafford 1992[31]	8M	65	CHO mix	7.0	TT	5 km	184	2.1	0.8
Wilber 1992[32]	10M	60	Exceed	7.0	TTE	80%	79	2.7	0.9
Zachwieja 1992[33]	8M	65	CHO mix	9.5	TT	15 min	90	3.3	1.5
Zachwieja 1992[33]	8M	65	CHO mix	9.5	TT	15 min	90	2.8	1.5
Cole 1993[34]	10M	60	CHO mix	6.0	TT	15 min	111	0.7	1.0
Cole 1993[34]	10M	60	CHO mix	8.3	TT	15 min	111	0.6	1.0
Cole 1993[34]	10M	60	CHO mix	8.3	TT	15 min	111	0.6	1.0

Table 1. Study-estimate characteristics, shown in chronological order by year and alphabetical order within years.

Reference	Sample size	VO_2 max[a]	Supplement	CHO+ protein (%)[b]	TT or TTE	Exercise protocol[c]	Exercise duration (min)[d]	Power effect (%)[e]	Power SE (%)[f]
Nishibata 1993[35]	7M	50	Glucose	10	TTE	73%	98	-0.8	1.1
Tsintzas 1993[36]	4M,3F	64	CHO mix	5.0	TT	30 km	131	2.3	0.6
Widrick 1993[37]	8M	58	CHO mix	9.1	TT	70 km	119	1.8	1.2
Bacharach 1994[38]	12M	68	CHO mix	6.4	TT	500 pedal revs	63	2.2	0.7
Bacharach 1994[38]	12M	68	CHO mix	10	TT	500 pedal revs	63	3.0	1.0
Chryssanthopoulos 1994[39]	5M,4F	63	Glucose	25	TTE	70%	121	1.5	0.7
Anantaraman 1995[40]	3M,2F	57	Glucose polymers	10	TT	60 min	60	10.5	2.8
Anantaraman 1995[40]	3M,2F	57	Glucose polymers	10	TT	60 min	60	7.0	2.8
Ball 1995[41]	8M	62	Glucose polymers	7.0	TT	30 s	49	5.6	1.6
Below 1995[42]	8M	63	CHO mix	5.9	TT	~10 min	87	0.8	1.2
Below 1995[42]	8M	63	Maltodextrin	39.5	TT	~10 min	88	1.0	1.2
El-Sayed 1995[43]	9M	61	Glucose	7.5	TT	10 min	38	4.5	1.1
Kang 1995[44]	7M	62	Gatorade	6.0	TTE	71%	154	2.8	1.1
Tsintzas 1995[45]	7M	54	CHO mix	5.5	TT	42.2 km	194	2.1	0.8
Tsintzas 1995[45]	7M	54	CHO mix	6.9	TT	42.2 km	194	0.8	0.8
Madsen 1996[46]	9M	63	Glucose	5.0	TT	100 km	160	-0.2	2.2
Madsen 1996[46]	9M	63	Glucose+protein	5.5	TT	100 km	160	2.2	2.2
Maughan 1996[47]	12M	59	Glucose	3.6	TTE	70%	101	2.6	0.9
Maughan 1996[47]	12M	59	Glucose	1.6	TTE	70%	101	1.3	0.9
McConell 1996[48]	8M	69	CHO mix	7.0	TT	15 min	92	4.3	1.2
McConell 1996[48]	8M	69	Glucose polymers	7.0	TT	15 min	92	1.8	1.2
Tsintzas 1996[49]	10M	57	CHO mix	3.5	TTE	70%	110	2.0	0.8
Tsintzas 1996[49]	10M	57	Lucozade	4.5	TTE	70%	110	1.6	0.8
Tsintzas 1996[50]	8M	57	CHO mix	5.5	TTE	70%	104	3.9	1.1
El-Sayed 1997[51]	8M	67	Glucose	8.0	TT	60 min	60	3.0	1.2
Jeukendrup 1997[52]	17M,2F	74	Isostar	7.6	TT	1039 kJ	60	2.2	0.6
Millard-Stafford 1997[53]	12M	66	Gatorade	6.0	TT	1.6 km	57	1.6	0.7
Millard-Stafford 1997[53]	12M	66	Powerade	8.0	TT	1.6 km	57	1.9	0.7

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Reference	Sample size	VO_2 max[a]	Supplement	CHO+ protein (%)[b]	TT or TTE	Exercise protocol[c]	Exercise duration (min)[d]	Power effect (%)[e]	Power SE (%)[f]
Jeukendrup 1998[54]	7M	74	CHO mix	10.5	TT	271 kJ	33	0.7	1.5
Kovacs 1998[55]	15M	60	CHO mix	6.8	TT	1083 kJ	62	1.0	0.8
Palmer 1998[56]	11M,3F	67	Energade	6.8	TT	20 km	28	0.1	0.9
Sugiura 1998[57]	8M	56	Glucose polymers	20	TT	40 s	105	4.9	0.9
Sugiura 1998[57]	8M	56	Fructose+protein	20	TT	40 s	105	3.6	1.0
Jarvis 1999[58]	0M,10F	61	Exceed	7.0	TT	30 s	72	1.0	1.0
McConell 1999[59]	8M	67	CHO mix	8.0	TTE	69%	152	4.2	1.1
Angus 2000[60]	8M	65	Gatorade	6.0	TT	35 $kJ \cdot kg^{-1}$	178	7.2	2.1
Febbraio 2000[61]	7M	63	Lucozade	4.5	TT	7 $kJ \cdot kg^{-1}$	148	9.9	3.6
Febbraio 2000[61]	7M	63	Lucozade	4.5	TT	7 $kJ \cdot kg^{-1}$	148	4.0	3.6
Febbraio 2000[61]	7M	63	Lucozade	9.0	TT	7 $kJ \cdot kg^{-1}$	148	11.9	3.6
McConell 2000[62]	13M	66	Glucose	5.5	TTE	83%	70	-0.2	0.8
Mitchell 2000[63]	10M	55	CHO mix	8.0	TT	10 km	42	0.5	1.0
Mitchell 2000[63]	10M	55	CHO mix	8.0	TT	10 km	42	0.2	1.0
Mitchell 2000[63]	10M	55	Glucose	6.0	TT	10 km	42	0.5	1.0
Mitchell 2000[63]	10M	55	Banana	6.0	TT	10 km	42	0.8	1.0
Mitchell 2000[63]	10M	55	CHO mix	6.0	TT	10 km	42	0.3	1.0
Bishop 2001[64]	9M	53	Glucose	5.0	TTE	75%	77	3.2	1.0
Chinevere 2002[65]	9M	62	Polydextrose	7.3	TT	449 kJ	92	9.1	1.1
Chryssanthopoulos 2002[66]	10M	59	Lucozade	6.9	TTE	70%	112	1.8	0.9
Carter 2003[67]	7M	60	Glucose polymers	6.4	TTE	60%	123	4.0	1.4
Carter 2003[67]	8M	60	Glucose polymers	6.4	TTE	60%	51	3.3	1.3
Jentjens 2003[68]	9M	64	Glucose	5.0	TT	691 kJ	46	-1.1	1.1
Jentjens 2003[68]	9M	64	Glucose	15	TT	691 kJ	46	-0.8	1.1
Jentjens 2003[68]	8M	64	Glucose	40	TT	691 kJ	46	0.0	1.2
Desbrow 2004[69]	9M	65	Gatorade	6.0	TT	1053 kJ	63	0.0	1.1
Nikolopoulos 2004[70]	8M	66	Lucozade	6.4	TTE	85%	51	1.4	1.1
Saunders 2004[71]	15M	53	Accelerade[g]	9.1	TTE	75%	82	3.3	0.8

Table 1. Study-estimate characteristics, shown in chronological order by year and alphabetical order within years.

Reference	Sample size	VO_2 max[a]	Supplement	CHO+ protein (%)[b]	TT or TTE	Exercise protocol[c]	Exercise duration (min)[d]	Power effect (%)[e]	Power SE (%)[f]
Burke 2005[72]	10M	66	Powergel	17.1	TT	21 km	74	0.3	0.4
Van Nieuwenhoven 2005[73]	90M,8F	60	Sucrose	6.9	TT	18 km	78	-0.4	0.3
Gusbakti 2006[74]	10M	45	CHO mix	6.0	TTE	64%	66	2.9	0.6
Gusbakti 2006[74]	10M	45	CHO mix	12	TTE	64%	66	5.8	1.8
Romano-Ely 2006[75]	14M	60	CHO mix	9.3	TTE	70%	96	0.4	1.3
Van Essen 2006[76]	10M	63	Sucrose	6.0	TT	80 km	141	9.5	2.0
Van Essen 2006[76]	10M	63	CHO+protein	8.0	TT	80 km	141	8.2	1.6
Saunders 2007[77]	8M,5F	63	CHO+protein	9.1	TTE	75%	103	1.5	0.7
Abbiss 2008[78]	10M	62	Sucrose gel	25	TT	16.1 km	42	5.6	2.5
Abbiss 2008[78]	10M	62	Sucrose gel	25	TT	16.1 km	93	3.3	1.0
Campbell 2008[79]	8M,8F	62	Gatorade	5.6	TT	10 km	108	1.3	0.6
Campbell 2008[79]	8M,8F	62	CHO gel	5.6	TT	10 km	108	1.6	0.6
Campbell 2008[79]	8M,8F	62	Sport beans	5.6	TT	10 km	108	1.7	0.6
Currell 2008[80]	8M	65	Glucose	14.4	TT	983 kJ	82	7.8	1.1
Currell 2008[80]	8M	65	CHO mix	14.4	TT	983 kJ	82	14.9	1.6
Hulston 2008[81]	10M	66	Glucose	6.4	TT	847 kJ	77	2.6	0.9
Jeukendrup 2008[82]	12M	66	CHO mix	5.9	TT	16 km	25	-0.5	0.6
Osterberg 2008[83]	13M	56	CHO mix	6.0	TT	514 kJ	102	2.5	1.1
Osterberg 2008[83]	13M	56	CHO+protein	9.1	TT	514 kJ	102	1.4	1.1
Peake 2008[84]	10M	62	Sucrose gel	9.4	TT	16.1 km	41	0.1	0.9
Peake 2008[84]	10M	62	Sucrose gel	5.8	TT	16.1 km	88	3.1	1.3
Valentine 2008[85]	11M	53	CHO mix	7.8	TTE	75%	107	1.1	0.6
Valentine 2008[85]	11M	53	CHO mix	9.7	TTE	75%	107	1.4	0.6
Valentine 2008[85]	11M	53	CHO+protein	9.7	TTE	75%	107	1.9	0.6
Breen 2009[86]	12M	63	CHO+protein	6.8	TT	880 kJ	87	0.1	0.5
Hulston 2009[87]	10M	62	CHO mix	6.0	TT	847 kJ	107	5.7	1.5
Hulston 2009[87]	10M	62	CHO mix	6.0	TT	847 kJ	107	5.5	1.4
Lacerda 2009[88]	9M	56	Maltodextrin	6.0	TTE	66%	93	-0.3	1.0

Table 1. Study-estimate characteristics, shown in chronological order by year and alphabetical order within years.

Reference	Sample size	VO_2 max[a]	Supplement	CHO+ protein (%)[b]	TT or TTE	Exercise protocol[c]	Exercise duration (min)[d]	Power effect (%)[e]	Power SE (%)[f]
Robson-Ansley 2009[89]	7M	60	Maltodextrin	7.5	TT	90 min	90	4.6	0.9
Saunders 2009[90]	13M	61	CHO+protein	7.8	TT	60 km	135	0.9	0.9

CHO = carbohydrate. **TT** = time trial presented as test duration or workload. **TTE** = time-to-exhaustion test.

a Data for VO_2max (ml·kg^{-1}·min^{-1}) are adjusted to 100% males.
b Total concentration of carbohydrate plus protein; percent unit is grams per 100 ml of total fluid consumed.
c Data shown for TT are measures of work done, distance traveled or test duration; data for TTE are intensity expressed as percent of VO_2max.
d Exercise duration = test duration + (fractional utilization)·(preload duration), where fractional utilization is the endurance capacity used up by preload (see text).
e Effect on mean power in a non-preloaded time trial.
f Adjusted standard error of the effect (see text).
g Food or drink containing protein.

1.2 Data Extraction

1.2.1 Performance Measures

To perform a meta-analysis, the magnitudes of effects from all relevant studies need to be expressed in a common metric. The most appropriate metric for athletic performance is power output in a time trial, because the effect can then be applied directly to competitive performance.[91] We therefore converted effects on performance in time-to-exhaustion tests and preloaded time trials to effects on power output in non-preloaded time trials.

Effects on time to exhaustion can be converted into effects in a time trial when the relationship between power output and duration is known.[92] The exercise in all studies that qualified for inclusion with time-to-exhaustion protocols was performed at or below VO_2max. An appropriate relationship between power and duration for such exercise is that of Leger et al.[93]: $P = a - b \cdot \ln(T)$, where P is the power expressed as percent of VO_2max, T is the duration, and a and b are constants for a given individual and mode of exercise. We assumed that this relationship could be applied with mean values of a and b for the subjects in a given study, which we derived by solving for a and b using values of power and duration for two intensities of exercise. The first intensity was given by the study itself: the time to exhaustion in the control condition at the given percent of

VO_2max. We used VO_2max as the second intensity, and we estimated the time to exhaustion at this intensity by regressing mean values of VO_2max against log of mean time to exhaustion from the studies reviewed by Billat and Koralzstein.[94] These studies provided values differing in gender (females, males) and exercise mode (cycling, running), but there appeared to be little effect of gender or mode on the regression. We concluded there was no need to adjust for gender and mode in predicting time to exhaustion at VO_2max. The values of a and b for the given study were then used to convert the times to exhaustion in control and treatment conditions into the ratio of power outputs in equivalent time trials using the relationship $P_t/P_c = (a - b \cdot \ln(T_c))/(a - b \cdot \ln(T_t))$, where c and t are control and treatment conditions. The percent effect of the supplement on time-trial power was then calculated as $100 \cdot (P_t/P_c - 1)$. We performed a sensitivity analysis to investigate the effect of error in the prediction of time to exhaustion at VO_2max equal to twice the standard error of the estimate. We observed ~5% change in the effect on time-trial power (e.g., 1.9% became 2.0%), so we are confident that this approach to converting effects on times to exhaustion into effects on time-trial times is trustworthy.

Effects on performance time in time trials were first converted to effects on mean power output by using the power-speed relationship $P = k \cdot S^x = a \cdot (D/T)^x$ where P is power, S is speed, D is distance, T is performance time, and k and x are constants.[91] Thus $P_t/P_c = (D_t/D_c)^x$ or $(T_c/T_t)^x$. (Performance in time trials that was measured as power output or work done did not require this conversion.) The constant x is 1.0 for running, but x varies between cycle ergometers, depending on the way they simulate distance traveled. For the Monark, x = 1.0.[90] For the Velodyne, x = 2.8; for the Velotron, x = 2.0 and 2.5 for time trials that include and do not include climbs (C.D. Paton and W.G. Hopkins, unpublished observations). A value of x was not available for the Politecnica, so we used the power-speed relationship $P = 9.65 \cdot S - 86.7$.[91] The approach for then converting the performance effect on power in a preloaded time trial into an effect in a non-preloaded time trial was novel and based on the assumption that the factor increase in error of measurement produced by a preload would apply also to the effects on performance. The conversion involved the following steps: estimate the typical (standard) error of measurement in all time trials where an exact p-value or confidence limits were

provided; estimate the fraction of endurance capacity utilized in the preload; derive the relationship between error and fractional utilization; use the relationship to predict the factor reduction in the error for a study without a preload (fractional utilization = 0); and finally, for all time trials, use this factor to convert the effect with the preload into an effect without a preload. The typical error of measurement was calculated as SEM·√(sample size)/√2, where SEM was the standard error of the mean effect estimated from the exact p-value and/or confidence limits via the t-statistic and its degrees of freedom (sample size – 1). The fractional utilization was calculated as (duration of preload)/(time to exhaustion at the preload intensity); time to exhaustion at the preload intensity was assumed to be dependent on VO_2max and was calculated with the Leger equation from estimates of a and b, which we derived by regressing values of a and b against values of VO_2max from the time-to-exhaustion studies (see previous paragraph). For the relationship between error and fractional utilization, we started with fractional utilization as a linear predictor of log(error), but we found substantial improvement in the prediction by including log(duration) interacted with fractional utilization as an extra predictor.

A meta-analysis also requires calculation of a standard error for each study-estimate. For studies that provided an exact p-value or confidence limits, we calculated the standard error for the effect on mean power in the equivalent non-preloaded time trial via the t-statistic and its degrees of freedom. Each of these studies also provided an estimate of the typical error for mean power in a time trial via the relationship between typical error and standard error (see previous paragraph). The typical error from the time-to-exhaustion studies was then averaged (via variances), allocated to time-to-exhaustion studies that did not report an exact p-value or confidence limits, and the standard error was calculated via the relationship between typical and standard error. For time-trial studies that did not report an exact p-value or confidence limits, the typical error predicted for zero fractional utilization in the relationship between error and fractional utilization (see previous paragraph) was used to calculate the standard error for the estimate of mean power in non-preloaded time trials.

1.2.2 Study Characteristics

Table 2 shows the mean study and study-estimate characteristics. VO_2max was included as a predictor in the meta-analytic model and was adjusted to the value expected for 100% males in studies of cyclists via a preliminary multiple linear regression in which sex and mode of exercise (running or cycling) were linear predictors of VO_2max (without interaction). In this preliminary analysis, and in the main meta-analysis, sex was coded as a variable with values equal to the fraction of males in the sample. All food and fluid consumed after the last meal before exercise was combined into an average supplement composition, and this supplement was assumed to be distributed equally across the boluses. The meta-analysis included the timing of intake of the first supplement bolus before the start of exercise, coded as a quadratic to take into account the fact that a supplement loses its effectiveness if taken too long before exercise. We included the frequency of supplement ingestion as the number of boluses divided by the time from intake of the first bolus to the end of exercise, on the assumption that equal boluses were ingested at equal intervals. Exercise duration for times to exhaustion was included as the log transformation of the test duration of the placebo treatment; for time trials, exercise duration was adjusted to include any preload by adding (fractional utilization)·(preload duration) to the test duration before log transformation. Blinding of the treatment was coded as a variable with values 0 or 1.

Table 3 shows the supplement ingestion regimes for the study-estimates. The measures that were included in the analysis as predictors were: the total percent concentration of carbohydrate and protein (grams of carbohydrate plus protein per 100 ml of total fluid ingested); the rates of ingestion of each of glucose, sucrose, fructose, and glucose polymers (grams per kilogram of body mass per hour, where *hour* referred to the time measured from the first ingestion of carbohydrate to the end of exercise); and the inclusion of salt (NaCl), coded as 0 or 1.

We were able to obtain some data missing from the manuscripts by emailing authors. Where we could not retrieve data, we derived values as follows. Missing data for VO_2max were assigned the mean VO_2max from other studies with similar subjects. Studies without a stated ambient temperature were assigned 21.3ºC, which was the average for all performance tests that appeared to be performed at normal room

temperatures. Missing data for supplement characteristics were either retrieved from the manufacturer's website or were assigned the average of all supplements with similar carbohydrate concentration. Where data on salt inclusion were missing, we assumed that commercially available supplements contained salt, and that supplements that were made in a lab did not. A spreadsheet containing individual values of the characteristics summarized in Tables 2 and 3 can be obtained from the authors.

Table 2. Study and study-estimate characteristics. Data are either counts, mean ± SD, or proportions (%).

	All studies		Studies with SE<1.25	
	Time trials	Time to exhaustion	Time trials	Time to exhaustion
Study characteristics				
Number of studies	56	32	44	29
Estimates per study	1.8 ± 0.9	1.6 ± 0.9	1.7 ± 1.0	1.6 ± 0.9
Sample Size	11.7 ± 12.1	9.2 ± 2.7	12.6 ± 13.5	9.3 ± 2.6
Males (%)	93	95	92	94
Mean body mass (kg)	72.1 ± 4.1	70.6 ± 4.6	71.9 ± 4.1	70.8 ± 4.1
Mean VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)[a]	61.6 ± 5.3	58.3 ± 6.0	61.4 ± 5.3	58.2 ± 6.3
Cycling (%)[b]	84	81	82	83
Fasting period (h)[c]	7.4 ± 4.3	9.7 ± 3.9	7.6 ± 4.2	9.8 ± 3.9
Ambient temperature (ºC)	22.5 ± 3.9	21.8 ± 3.1	23.1 ± 4.1	21.3 ± 2.1
Intake-time first bolus (h)[d]	0.5 ± 0.7	0.5 ± 0.6	0.5 ± 0.8	0.5 ± 0.6
Number of boluses	5.5 ± 3.0	5.7 ± 3.4	5.0 ± 3.0	5.7 ± 3.5
Boluses per hour[e]	3.0 ± 1.4	3.0 ± 1.7	2.8 ± 1.5	2.9 ± 1.7
Preload intensity (%)[f]	68 ± 7	-	68 ± 7	-
Preload duration (min)[f]	89 ± 30	-	84 ± 30	-
Fractional utilization[g]	0.73 ± 0.42	-	0.73 ± 0.45	-
Test duration (min)[h]	53 ± 50	103 ± 41	47 ± 44	106 ± 41
Exercise duration (min)[i]	92 ± 41	103 ± 41	85 ± 41	106 ± 41
Study-estimate characteristics				
Number of study-estimates	103	52	75	47
Blinded (%)[j]	90	65	91	62
Change in power (%)[k]	2.7 ± 3.1	1.7 ± 1.8	1.7 ± 2.0	1.5 ± 1.6
Standard error[l]	1.2 ± 0.7	1.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.2
Typical error[l]	2.7 ± 1.1	2.0 ± 0.5	2.1 ± 0.4	1.9 ± 0.3

SE = adjusted standard error of the effect (see text).
a Adjusted to 100% males.
b Percent of studies that used cycling exercise.
c Time between last feeding defined by the researchers as a meal and start of exercise.
d Measured to the beginning of the adjusted period of exercise.
e Number of boluses/actual time from first bolus to end of exercise.
f For 34 and 26 preloaded time trials in all studies and studies with SE<1.25% respectively.
g Endurance capacity used up by preload (see text).
h For the placebo treatment.
i Test duration + (fractional utilization)·(preload duration).
j Percent of study-estimates from placebo-controlled (at least single-blind) designs.
k After conversion into non-preloaded time trial (see text).
l Adjusted for fractional utilization = 0 (see text).

Table 3. Supplement-ingestion regimes for the study-estimates. Data are mean ± SD or proportions (%).

	All studies		Studies with SE<1.25	
	Time trials (n=103)	Time to exhaustion (n=52)	Time trials (n=75)	Time to exhaustion (n=47)
CHO+protein (%)[a]	9.6 ± 6.9	11.6 ± 8.7	10.0 ± 7.6	11.7 ± 9.0
Volume (L)	1.3 ± 0.7	1.5 ± 1.5	1.1 ± 0.6	1.5 ± 1.6
Glucose ($g \cdot kg^{-1} \cdot h^{-1}$)	0.4 ± 0.5	0.4 ± 0.7	0.3 ± 0.5	0.5 ± 0.7
Sucrose ($g \cdot kg^{-1} \cdot h^{-1}$)	0.3 ± 0.5	0.3 ± 0.6	0.2 ± 0.4	0.2 ± 0.5
Fructose ($g \cdot kg^{-1} \cdot h^{-1}$)	0.2 ± 0.3	0.3 ± 1.0	0.2 ± 0.3	0.3 ± 1.0
Glucose polymers ($g \cdot kg^{-1} \cdot h^{-1}$)	0.4 ± 0.8	0.5 ± 0.9	0.4 ± 0.8	0.5 ± 0.9
Protein ($g \cdot kg^{-1} \cdot h^{-1}$)	0.01 ± 0.07	0.04 ± 0.10	0.01 ± 0.05	0.03 ± 0.10
Protein included (%)	5.8	13	5.3	15
Salted (%)[b]	59	56	55	51

SE = adjusted standard error of the effect (see text). **CHO+protein** = total concentration of carbohydrate plus protein.

a Percent unit is total grams per 100 ml of total fluid consumed.

b Percent of study-estimates with salt in the supplement.

1.2.3 Publication Bias and Outliers

Published effects are on average larger than true values, owing to the misuse of statistical significance as a criterion for publication.[95] To reduce the effects of publication bias, we plotted the standard error of each study-estimate versus the t-value for the solution for the between-study random effect (Figure 1).[96] A line was the drawn at a value for the standard error that divided the scatter into a symmetrical plot on the left and an asymmetrical plot on the right. Asymmetrical scatter is very likely the result of a publication trend towards positive effects, so the meta-analysis was also performed only for those study-estimates falling on the left of the line. T-values of the study-estimates were also used to assess the presence of outliers.[96]

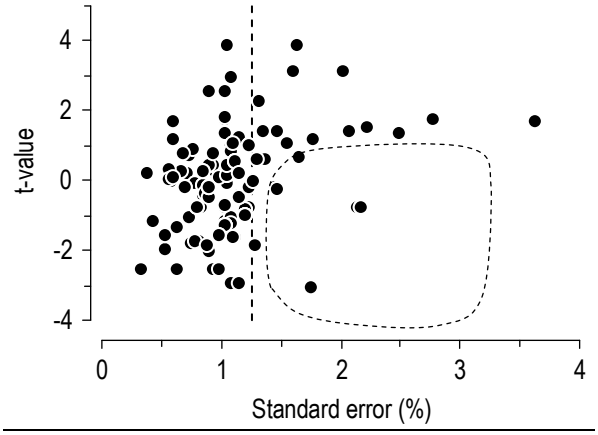

Fig. 1. Scatter-plot to investigate outlier studies and publication bias. The t-value of the solution of the between-study random effect is plotted against the standard error for the study-estimate. Dashed line at a standard error of ~1.25% divides the plot into a region with symmetric scatter (in the vertical direction) to the left and a region to the right where a dearth of t-values within the dashed curve is apparent.

1.3 Meta-analytic Model

The meta-analyses were performed with the mixed linear modeling procedure (Proc Mixed) in the Statistical Analysis System (Version 9.2, SAS Institute, Cary, NC). Percent effects on mean power output were converted to factors (= 1+effect/100), log transformed for the analysis, then back transformed to percents. The fixed effects in the meta-analytic model consisted of binary and continuous predictors representing study characteristics. The binary predictors were mode of exercise (1=cycling, 0=running), type of performance test (1=time trial, 0=time to exhaustion), blinding (1=blind), salt in drink (1=included), and variables for each level (low, moderate and high) of each carbohydrate, protein, and concentration of carbohydrate plus protein, as defined in Table 4. The continuous predictors were ambient temperature, adjusted VO_2max, time of ingestion of first bolus before exercise (as a quadratic), fasting period before exercise, fraction of males in the sample, number of boluses per hour, the amount of glucose, sucrose, fructose and glucose polymers ingested, and the total concentration of carbohydrate plus protein. Values of the predictor variables characterizing the composition of the carbohydrate supplement were the values in the supplement treatment minus the values in the control or reference treatment. In a further analysis, we included a predictor to account for the possibility of a synergistic effect of co-ingesting two or more carbohydrates. The

predictor for synergism was coded 0 for ingestion of one carbohydrate source, and 1 for ingestion of two or more carbohydrate sources. The random effects in the model specified within-study SD and between-study SD; negative estimates for variances were allowed, and SD derived from negative variances were expressed as negative SD.

At the suggestion of a reviewer, we used the above mixed model to analyze the studies in two groups: those where ingestion began earlier than 15 min before exercise and those where ingestion began at 15 min or later. To allow for greater non-linearity in the effect of time of commencement of ingestion, we also modelled its effect as a cubic with the full data set.

Uncertainty in the meta-analyzed estimates is reported as 90% confidence limits. We made probabilistic magnitude-based inferences about the true values of outcomes, as described elsewhere.[96] In brief, an outcome was deemed unclear if its confidence interval overlapped thresholds for smallest worthwhile positive and negative effects; equivalently, effects were unclear if chances of the true value being substantially positive and negative were both >5%. The magnitude of a clear effect was reported as the magnitude of its observed value, sometimes with an assertion about the probability the true value was substantial. The thresholds for small, moderate, large and very large effects on performance were assumed to be 0.3, 0.9, 1.6 and 2.5 of the race-to-race within-athlete variability in competitive performance of elite athletes.[96] Although the meta-analyzed studies used mainly sub-elite athletes or active non-athletes, thresholds for elite athletes were used here, as in all previous studies using this approach to inferences, under the assumption that the effects will apply to elites and that medal winning by elites is more important than that by non-elites. The variabilities in mean power output for endurance running and cycling in races are ~0.8%[97] and ~3.5%[98] respectively, giving smallest effects of 0.25% and ~1.0%. We chose 0.5% as a value to apply to high-intensity endurance sports generally; thresholds for moderate, large and very large effects were therefore 1.5%, 2.7% and 4.2%. The magnitudes of the effects of continuous predictors (VO_2max, ambient temperature, exercise duration, fasting period, rate and timing of bolus ingestion) were assessed for an increase of ~2 between-study SD of the predictor (or of the log of the predictor, for those that were log-transformed for the analysis).[96] By

analogy with this approach, we assessed the magnitude of the between-study random effect for twice its SD.

Results

Figure 1 shows the plot that was used to assess the presence of outliers and publication bias. The plot showed an asymmetrical scatter for studies with an error >~1.25%. The meta-analysis was then repeated for studies with a standard error <1.25% to reduce publication bias. None of the t-values was considered sufficiently large to warrant exclusion of the study-estimate.

Table 4 shows the components of the meta-analytic model expressed as additive percent effects (±90% confidence limits) on mean power in a non-preloaded time trial. Effects for supplement composition were generally reduced by up to ~1.5% after adjusting for publication bias. Combining these effects into a meta-analyzed performance effect reduced the effect by up to 2% (e.g., from 5% to 3%). Here we describe the effects only for studies with a standard error <1.25%.

The effects shown for the supplement composition in Table 4 have to be added to an intercept for a given condition, which can be illustrated with the following example. The intercept is 0.6% for a male subject with a VO_2max of 60 $ml \cdot min^{-1} \cdot kg^{-1}$ performing a 100-min cycling time trial with no preload at 22ºC after a 4-h fast who knowingly ingests 1 $L \cdot 70\,kg^{-1} \cdot h^{-1}$ of a supplement without salt in six boluses per hour, with the first bolus ingestion at the start of exercise. For a supplement providing 1.1 $g \cdot kg^{-1} \cdot min^{-1}$ glucose polymers and 0.1 $g \cdot kg^{-1} \cdot min^{-1}$ protein in a drink with an 8% concentration of carbohydrate plus protein, the meta-analyzed effect on performance is 5.3% (90% confidence limits, ±2.6%). The best effect for this condition was a performance increase of 6.5% (±2.5%) for a supplement providing ~0.7 $g \cdot kg^{-1} \cdot h^{-1}$ glucose polymers, ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ fructose and ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ protein. Carbohydrate supplementation regimes may also impair performance. The largest impairment (-2.1%, ±2.3%) was found for a >15% fructose supplement providing >0.5 $g \cdot kg^{-1} \cdot h^{-1}$ ingested as 1 bolus per hour with the first bolus ingestion at the start of exercise.

Table 4. Components of the meta-analytic model expressed as additive percent effects (±90% confidence limits) on mean power in a non-preloaded time trial. Effects shown are for a difference between levels (e.g. female – male) or for an increase of ~2 SD (e.g. VO_2max per 10 ml·min^{-1}·kg^{-1}).

	All studies	Studies with SE<1.25%
Supplement composition		
Intercept[a]	(0.8; ±1.9	0.6; ±1.8
CHO+protein[b]		
Low	1.1; ±1.9	1.1; ±1.7
Moderate	1.2; ±1.8	1.1; ±1.6
High	0.5; ±2.0	0.3; ±1.7
Glucose[c]		
Low	-0.1; ±0.7	-0.2; ±0.6
Moderate	0.4; ±1.0	-0.6; ±0.9
High	-0.9; ±1.3	-0.3; ±1.2
Sucrose[c]		
Low	-0.2; ±0.6	-0.2; ±0.5
Moderate	0.3; ±0.9	-0.7; ±0.8
High	1.4; ±1.7	-0.2; ±1.5
Fructose[c]		
Low	0.4; ±0.6	0.3; ±0.6
Moderate	-1.2; ±1.3	-2.2; ±1.1
High	-1.7; ±2.3	-2.2; ±2.1
Glucose polymers[c]		
Low	-0.3; ±0.7	-0.2; ±0.7
Moderate	0.4; ±1.0	0.0; ±1.0
High	1.5; ±1.4	0.6; ±1.4
Protein[d]		
Low	0.1; ±1.1	0.2; ±1.0
Moderate	1.9; ±1.4	1.1; ±1.2
High	1.0; ±3.9	0.6; ±3.3
Study characteristics		
Female – male	0.3; ±2.3	-0.4; ±2.0
VO_2max[e] (per 10 ml·min^{-1}·kg^{-1})	-0.4; ±0.7	-0.1; ±0.4
Ambient temp (per 10ºC)	-0.5; ±0.9	-0.5; ±0.8
Running – cycling	0.0; ±1.0	-0.2; ±0.9
Time to exhaustion − time trial	-1.0; ±0.8	-0.5; ±0.8
Exercise duration[f] (per 3-fold increase)		
Low CHO+protein[c]	1.1; ±1.7	1.2; ±1.7
Moderate CHO+protein	1.2; ±0.7	0.2; ±0.7
High CHO+protein	-0.4; ±0.8	-0.5; ±0.7
Fasting (per 9 h)	0.5; ±0.9	1.1; ±0.8
Blind − not blind	0.2; ±1.0	0.1; ±1.0

Inclusion of salt	-0.4; ±0.6	-0.3; ±0.6
Boluses (per 3 h^{-1})	0.9; ±0.9	0.7; ±0.8
Time of first bolus ingestion[g]		
Linear (per h)	1.4; ±0.9	1.1; ±0.8
Quadratic (per h^2)	-0.3; ±0.2	-0.1; ±0.2
Maximum effect (at ~4 h)	1.4; ±2.3	2.4; ±2.1
Random Variation		
Between-study SD	1.7; ±0.4	1.4; ±0.3
Within-study SD	0.4; ±0.6	-0.4; ±0.5

SE = adjusted standard error of the effect (see text).
CHO+protein = total concentration of carbohydrate plus protein.
a Intercept for a male subject with a VO_2max of 60 ml·min^{-1}·kg^{-1} performing a 100-min cycling time trial at 22ºC after a 4-h fast, who knowingly ingests 1 L·70kg^{-1}·h^{-1} of a supplement without salt as three boluses per hour, with the first bolus ingestion at the start of exercise.
b Thresholds (%): 0<low≤5<moderate≤15<high.
c Thresholds (g·kg^{-1}·h^{-1}): 0<low≤0.25<moderate≤0.50<high.
d Thresholds (g·kg^{-1}·h^{-1}): 0<low≤0.125<moderate≤0.25<high.
e Adjusted to 100% males.
f Test duration + (fractional utilization)·(preload duration).
g Before the start of exercise.

Reductions in the performance effect of carbohydrate supplementation were possibly moderate with a supplement providing >0.25 g·kg^{-1}·h^{-1} fructose. Reductions were possibly small with an increase in ambient temperature of 10ºC and in time-to-exhaustion tests compared with time trials. A three-fold increase in exercise duration also reduced the performance benefit when supplements with high carbohydrate-plus-protein concentration were ingested; with low concentrations the performance benefit was moderately increased for longer exercise durations, but this effect was unclear. Increases in the effectiveness of carbohydrate ingestion on performance were likely to be small with an additional 9-h fast and with ingesting moderate rates of protein. Increases were possibly small with increasing the frequency of supplement ingestion by three boluses per hour and likely small-moderate with ingesting the first bolus not at the start of exercise but 1-4 h before exercise. Effects were likely trivial for including salt in the supplement and for subjects with a VO_2max higher by 10 ml·min^{-1}·kg^{-1}; effects of gender, exercise mode, exercise duration when supplements with low or moderate carbohydrate-plus-protein concentrations were ingested, and blinding of treatment were unclear.

The magnitude of the between-study random variation (interpreted as 2 between-study SD) was large and positive. The magnitude of the within-study random variation was small and negative, although its confidence interval allowed for trivial positive variation.

The estimate for synergism arising from co-ingestion of two or more carbohydrates had a negative trivial value; the effect was unclear but at most small (-0.3%, ±1.0%). The contributions of the components of the meta-analytic model in studies where ingestion began earlier than 15 min before exercise were very similar to those in the analysis of the full data set. A substantial difference was a greater contribution of consumption of multiple boluses (per 3 per hour: 1.9%, ±1.2%). The only other major difference was a moderate but unclear benefit of high sucrose ingestion (2.8%, ±4.5%). Contributions of components in studies where ingestion began ≤15 min before the start of exercise were generally less clear, but the combinations investigated for the full data set produced similar effects on performance and a similar effect of time of commencement of ingestion (over the range 15 min before exercise to 30 min after). In the analysis in which we modelled the effect of time of ingestion of first bolus as a cubic, the coefficient of the cubic was practically zero, the maximum still occurred at 4 h before exercise, and there was almost exactly the same gradual decrease between 4 h before exercise and 1 h post exercise.

Discussion

This study is the first meta-analytic review of the effects of acute carbohydrate supplementation on performance. To our knowledge, this meta-analysis is also first in our discipline to account for publication bias. This bias was substantial (up to 2% increase in the performance effect of supplements), which underscores the value of meta-analysis in providing more realistic performance effects of treatments than would be obtained in narrative reviews or from a researcher's impression of the effect of supplements. After adjusting for publication bias, the meta-analyzed performance effects of carbohydrate supplements ranged from clear improvements of ~6% to clear impairments of ~2%. The best supplement derived from the analysis provided ~0.7 $g \cdot kg^{-1} \cdot h^{-1}$ glucose polymers, ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ fructose and ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ protein. A possible explanation for this combination of carbohydrates is that the increase in carbohydrate oxidation rate with

ingestion of several varieties of carbohydrate helps the athlete to sustain exercise intensity (for review see Jeukendrup[2]). Our analysis did not provide evidence for a synergistic effect of ingesting several carbohydrate sources (other than a simple additive effect), although we cannot exclude a small positive or negative synergistic effect. The meta-analysis also shows that the best single source of carbohydrate consumed at a high rate is glucose polymers. This superiority is apparently not due to the lower osmolarity of glucose polymers, because osmolarity has little effect on gastric emptying and carbohydrate oxidation.[99] One possible explanation is that it may cause less gastro-intestinal distress compared with the other carbohydrates.[100] Inclusion of supplement osmolarity and gastro-intestinal distress in the meta-analytic model might have helped resolve this issue, but unfortunately we were unable to code osmolarity and gastro-intestinal distress for enough studies from the limited information provided by authors and manufacturers.

Recent studies, including some included in this meta-analysis, have provided evidence for higher rates of oxidation of carbohydrate and enhanced performance with ingestion of multiple forms of carbohydrate, mediated via multiple carbohydrate transporters in the intestinal epithelium.[2] Inspection of the effects of the different kinds of carbohydrate in Table 4 for the studies with SE<1.25% shows that most are negative, the only exceptions being high rates of glucose polymers and low rates of fructose (hence our recommendation above). Thus the meta-analysis provides only limited evidence for the benefit of ingestion of multiple forms of carbohydrate, namely fructose plus glucose polymers. Although the confidence limits allow for the possibility of benefits from including glucose and sucrose, it is possible that the higher carbohydrate oxidation rates with multiple forms of carbohydrate are not accompanied proportionally by enhanced performance. Our meta-analysis also suggests that more than low rates of consumption of fructose should be avoided. Indeed, the largest performance impairment derived from the analysis would occur with ingestion of a single moderate bolus of fructose at the start of exercise. Possible explanations for this harmful effect are gastro-intestinal distress[23] and the conversion of fructose to glucose, which may be too slow to maintain high carbohydrate oxidation rates in the later stages of exercise.[101]

Whether or not protein should be included in a carbohydrate supplement is a topical issue. The effects of protein in this meta-analysis are effects to be added to those of carbohydrate, which was always ingested with any protein in the analyzed studies. Our meta-analysis suggests that the inclusion of moderate amounts of protein in a carbohydrate supplement substantially increases performance. From the perspective of energy intake, moderate rates of protein ingestion are similar to low rates of carbohydrate ingestion. Effects for these corresponding levels of protein and carbohydrate in Table 4 suggest that protein is potentially more effective than any of the carbohydrates and may therefore mediate its effect on performance via more than simply provision of energy. One possibility is a placebo effect with protein, because it is difficult to blind subjects to the addition of protein in a carbohydrate beverage. The overall placebo effect in the meta-analysis was trivial, but there is substantial uncertainty in the estimate, and in any case subjects might get an extra placebo effect in protein-supplement studies from second-guessing that they have received a treatment that is potentially beneficial.

A possible explanation for the reduction in the performance benefit with ingesting high concentrations of carbohydrate and protein is slower gastric emptying,[102] which would limit the rate of carbohydrate absorption. High concentrations of carbohydrate and protein could also compromise fluid balance, either because the high concentration represents inadequate fluid intake or because the high osmolarity could draw fluid from the circulation into the gut.[103]

The trivial effect of salt inclusion in the supplement was unexpected: researchers have demonstrated that salt increases the net intestinal absorption rate of carbohydrate and that it promotes retention of ingested fluids,[104] which should have a beneficial effect on performance. Our variable representing salt was crude because of poor reporting in many of the studies. We expect that inclusion of salt in a supplement is beneficial provided it is not present at high concentrations.

Various study characteristics clearly moderated the performance effect of carbohydrate supplements. A possible mechanism for the reduction in the performance effect of carbohydrate with an increase in ambient temperature is the increase in core temperature that has been demonstrated during high-intensity exercise with carbohydrate ingestion compared with placebo[105]: this increase in core temperature may cause an

earlier onset of fatigue in a warmer environment; another possible mechanism is a decrease in the contribution of exogenous carbohydrate to substrate utilization at higher environmental temperatures.[106] Both of these mechanisms could arise from changes in blood flow to gut, skin and muscle.

The effect of the timing of first bolus ingestion appeared to be modelled adequately with a quadratic: the cubic produced little additional difference in the effects of time of ingestion before versus during exercise, even though there could be substantial physiological differences between ingesting carbohydrates either before or during exercise. If more studies were available where ingestion began immediately before or during exercise, meta-analysis might reveal different effects for some carbohydrates and/or protein compared with their effects when ingestion begins earlier. However, the smaller performance gains from such ingestion regimes would make further investigation of this issue academic.

The benefits with increasing the frequency of bolus ingestion and with ingesting the first bolus of the supplement earlier than at the start of exercise may have several explanations: a reduction in gastro-intestinal distress, a change in glucose metabolism mediated by changes in the insulin response,[107], or possibly even effects of ongoing stimulation of carbohydrate receptors in the mouth.[108] A longer fast increased the performance effect of carbohydrate ingestion, probably because of a reduction in hepatic glycogen stores. This finding is in agreement with the ~1% enhancement arising from an additional 9-h fast on performance in a 21-km running time trial in Wong et al.,[109] a study we could not include in the analysis because of its design. Although the effect had considerable uncertainty, a three-fold increase in exercise duration increased the performance benefit of a supplement when drinks of low carbohydrate-plus-protein concentration were ingested. Gastro-intestinal distress and/or a delay in gastric emptying may explain why the opposite (and clear) effect was seen for supplements with high concentrations. Low concentration drinks may provide sufficient energy to maintain high carbohydrate oxidation rates, and the longer the test, the more performance may depend on this provision of energy. There may be some subtle confounding effects of exercise duration in time-to-exhaustion tests arising from substantial performance enhancements and impairments; the meta-analytic model did not account for any differences in energy

consumed with the substantial changes in exercise duration that can occur with this protocol. Moderating effects of gender, and blinding were unclear, which indicates that more research is necessary to investigate these predictors. Researchers should be cautious to extrapolate results of this meta-analysis to high-intensity short-duration tests, as there was only one test with an exercise duration (adjusted to zero preload) <25 min (Table 1). Carbohydrate supplementation in short-duration exercise is unlikely to benefit performance via increased carbohydrate oxidation, but other mechanisms may be involved, including activation of brain regions via carbohydrate receptors in the mouth.[108] Similarly the longest duration of exercise (including pre-loads) in the meta-analysis was 3.3 h (Table 1), so extrapolation to ultra-endurance exercise is also inappropriate, although clearly there will be beneficial effects for such exercise from carbohydrate supplementation.

The meta-analysis did not include studies with substantial rest-intervals either because of difficulties with calculation of a fractional utilization or because of interactions with recovery from exercise. All performance tests included in the analysis consisted of continuous cycling or running. The small difference in the performance effect of supplements in time trials vs time-to-exhaustion tests is probably an artefact of the ways we have converted time-to-exhaustion tests and preloaded time trials into non-preloaded time trials. In view of the complexity of these conversions, the small magnitude of this difference is reassuring.

Insights into the practical application of the findings of the meta-analysis can also be gleaned from a consideration of the between- and within-study standard deviations (bottom of Table 4). These standard deviations represent unexplained variation in the mean effect of the protocol from study to study and within a study; as such, their magnitude is the typical deviation from the meta-analyzed mean effects that a researcher or practitioner can expect to experience in his or her setting. The unexplained variation arises in several ways: poor measuring or reporting of covariates; unknown covariates modulating the effect on performance; and a meta-analytic model that does not fully capture the complexity of the underlying reality, including non-linear effects and interactions. The large between-study difference means that researchers and practitioners might not see beneficial effects of some carbohydrate protocols in their setting. The

magnitude of the within-study random variation was negative, which implies variation between estimates within studies being smaller than expected from the standard errors of the estimates; this outcome is probably a consequence of our estimates of measurement error being too large. It follows that our estimates of uncertainty (confidence intervals) are in general conservative, and that our outcomes are trustworthy, as least as far as random sampling variation is concerned.

It is also important to understand that some individual athletes may obtain no benefit or even impairment in performance from carbohydrate ingestion, even with those protocols that are clearly beneficial. Meta-analysis cannot address the question of individual responses to treatments until researchers provide complete inferential information about experimental and control groups in the form of confidence limits, exact p-values, or best of all, standard deviations of change scores. In crossover studies researchers would need to include an extra trial in one or more of the treatment conditions to permit estimation of individual responses.[110]

We have simplified the various protocols in the studies to address the obvious questions that an athlete would ask about energy supplements for an endurance competition: what should I take, when do I start, and how often should I take it? This meta-analysis provides a new best-practice that researches can use to further improve supplementation protocols.

Conclusion

Carbohydrate supplements can have large performance benefits in endurance exercise. A good supplementation regime is to ingest carbohydrate before and during exercise in many boluses with the first bolus up to 4 h before the start of exercise. Supplements containing high concentrations of carbohydrate or more than small amounts of fructose should be avoided. A supplement providing ~0.7 $g \cdot kg^{-1} \cdot h^{-1}$ glucose polymers, ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ fructose and ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ protein may give the largest ergogenic effect. This meta-analysis did not account for individual responses: individual athletes may obtain no benefit or even impairment in performance with carbohydrate ingestion. In future, research should focus on females, short-duration exercise, ultra-endurance exercise,

better reporting of inferential statistics, and in cross-over studies inclusion of additional trials.

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CHAPTER 5

General Summary

This PhD consists of four research projects that were performed to investigate the effect of specific acute supplementation protocols on performance and/or recovery from training. Figure 1 shows a time line with best practice inferred from this research. Other acute and chronic strategies may be added on the basis of other research (e.g., sodium bicarbonate, beta-alanine, creatine). Here, I will summarize the outcomes of our research and describe the rationale behind the time line.

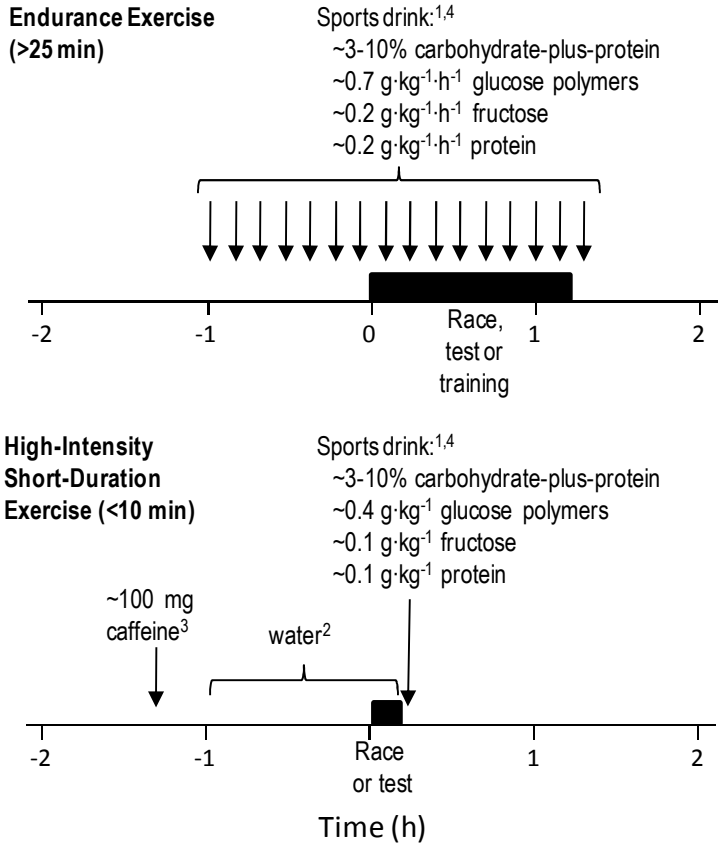

Figure 1—Best practice inferred from this PhD for acute supplementation protocols for performance and recovery from exercise. Superscripts refer to corresponding studies/chapters.

In Study 1 we have provided some evidence that consuming carbohydrate during and carbohydrate-protein immediately after exercise in a 2-h high-intensity swim session induces better recovery in plasma creatine kinase and salivary IgA compared with consuming water during exercise and carbohydrate-protein immediately after, and compared with consuming only carbohydrates during and immediately after exercise. These effects may indicate reduced muscle damage and better mucosal immunity in the upper respiratory tract. We have also demonstrated that the inclusion of protein in a carbohydrate supplement reduces inflammatory responses. We have therefore advised athletes to consume a blend of carbohydrate and protein during and immediately after exercise for recovery purposes. As shown in the meta-analysis (Study 4), this nutritional strategy may also enhance endurance performance. The best supplement inferred from the meta-analysis consisted of a ~3-10% carbohydrate-plus-protein drink providing ~0.7 $g \cdot kg^{-1} \cdot h^{-1}$ glucose polymers, ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ fructose and ~0.2 $g \cdot kg^{-1} \cdot h^{-1}$ proteinin multiple boluses before and during exercise. In this first meta-analytic review of the effects of acute carbohydrate supplementation on performance, we have focused on obvious questions that an athlete would ask about energy supplements for an endurance competition: what should I take, when do I start, and how often should I take it? This study took us almost two years, but this substantial amount of work has now provided a new best-practice that researches and sports beverage companies can use to further improve supplementation products and strategies.

Figure 1 shows a different time line for performance tests with exercise duration >25 min and <10 min for two reasons: the meta-analysis included only one study of performance effects in a test <25 min, and in Study 2 we have provided some evidence of performance impairment in short-duration swim exercise with carbohydrate-protein ingestion before exercise. For this general summary, we have used the meta-analysis to generate an estimate for the performance effect of the supplementation regime used in Study 2. Assuming that the exercise duration (adjusted to zero preload) for the last test in the step test was ~6 min and that the swimmers had a VO_2max (adjusted to 100% males) of 64 $ml \cdot min^{-1} \cdot kg^{-1}$, the meta-analyzed performance effect was 2.6% (90% confidence limits ± 4.2%). The confidence limits accommodate the observed impairment of 0.9% in Study 2. The uncertainty in the generated estimate was large, presumably because of the

higher fraction of females in the sample and because of the extrapolation to short-duration exercise. We did not use the meta-analysis to estimate the effect of the supplement in the 100-m time trial (the other performance test used in Study 2), because it would have been inappropriate to estimate the effect for such a short-duration test. Caution is also required to extrapolate effects of the meta-analysis to swim exercise, because the analysis was based on cycling and running.

Researchers have argued that ingestion of high-glycemic supplements before exercise may cause earlier onset of fatigue during exercise because of surges in insulin (Coggan & Coyle, 1989b; Thomas et al., 1991). In contrast, the meta-analysis supports ingestion of carbohydrate supplements for a period of up to 4 h pre-exercise. Including insulin concentrations as a covariate in the meta-analysis may have resolved this issue, but investigating metabolic responses to carbohydrate ingestion was beyond the scope of the study. Researchers have demonstrated that insulin responses can be attenuated by ingesting the carbohydrate supplement in many small boluses (Burke et al., 1996) and by warm-up exercise (Brouns et al., 1989). In the absence of any other evidence this practice of ingesting carbohydrate prior to exercise of duration >25 min should be regarded as best practice. In Study 2, we have stated that a surge in insulin may have been responsible for the possible performance impairment in the 100-m time trial with ingestion of one bolus of a carbohydrate-protein sports drink 10 min before this time trial. Other explanations in terms of carbohydrate availability seem unlikely, because the timing of intake would not have allowed much of the carbohydrate to be absorbed, and the duration of exercise would have been too short.

In Study 3, we have provided an innovative methodological approach for monitoring acute effects of training and other interventions on athletic performance. We monitored performance in a squad of elite swimmers preparing for Olympic-qualifying trials and used mixed linear modeling of performance times and a novel coding method to demonstrate better performance in evenings vs mornings, better performance in competition vs training time trials, and better performance with caffeine. This method also provides a tool to quantify individual trends in performance with training.

The performance effect of a 100-mg dose of caffeine ingested 75 min before a training or competition time trial was ~1.3%. We observed that performance was not increased

with higher doses of caffeine, but this effect was unclear. More research is necessary to investigate the effects of chronic caffeine ingestion in training. It is not clear whether chronic caffeine ingestion would affect the adaptive response to training, and habituation may reduce the performance effect (Fisher et al., 1986). Until this research is done, we advise that caffeine is used only in important tests or races.

The following are some limitations of the thesis.

- The clinical and functional relevance of the findings in Study 1 is uncertain. We did not investigate the effect of the dietary treatments on functional recovery (performance), because the coaches were unwilling to include standardized performance tests. Further investigations focused on effects of dietary treatments on functional recovery are needed to advise athletes and coaches of best-practice.
- In Study 2, we had asked the athletes to verbally report presence or absence of gastro-intestinal distress. In the event, none was reported, but if we had measured gastro-intestinal distress on a Likert or similar scale, we may have gained a better understanding of potential mechanisms behind the performance impairments in the last step of the step test and in the time trial.
- In Study 3, most outcomes were clear but a larger sample size would have provided clearer outcomes about individual differences in responses and about the effect of increasing the dose of caffeine.
- In the meta-analysis (Study 4)…
    - We have performed substantial modeling to convert time-to-exhaustion tests and preloaded time trials into non-preloaded time trials, and to estimate standard errors. This complexity decreases the trustworthiness in the outcomes. We were also unable to include studies with substantial rest-intervals either because of difficulties with calculation of a fractional utilization or because of interactions with recovery from exercise.
    - There was substantial unexplained variation between outcomes in different studies arising from: poor measuring or reporting of covariates; unknown covariates modulating the effect on performance;

and a meta-analytic model that does not fully capture the complexity of the underlying reality. The large between-study difference means that researchers and practitioners might not see beneficial effects of some carbohydrate protocols in their setting.

In summary, we have provided some evidence for:

- better acute recovery from training with ingesting carbohydrate during exercise and carbohydrate-protein immediately after exercise (Study 1);
- possible substantial performance impairments with carbohydrate-protein ingestion in high-intensity short-duration exercise, implying only water should be consumed (Study 2);
- a substantial performance increase with caffeine, established with an innovative approach to design and analysis that can be applied to investigation of other factors affecting performance (Study 3);
- substantial beneficial and harmful effects on endurance performance with carbohydrate supplements, derived from a comprehensive, innovative meta-analysis (Study 4).

Future directions

More research is needed to investigate:

- effects of carbohydrate and carbohydrate-protein supplements on recovery from exercise, using designs that include testing of performance;
- performance effects of carbohydrate and carbohydrate-protein supplements in females, in high-intensity short-duration exercise, and with chronic ingestion of these supplements;
- effects of chronic caffeine intake.

We encourage athletes, sports scientists and coaches to estimate magnitudes of effects of treatments and individual responses to treatments with linear modeling of performance times.

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APPENDIX A

Poster for *Acute Effects of Nutritional Supplementation on Recovery from Training in Elite Swimmers*

This poster won the award for best poster presentation at the 2007 Sport and Exercise Science New Zealand conference in Hamilton.

Acute effects of nutritional supplementation on recovery from training in elite swimmers

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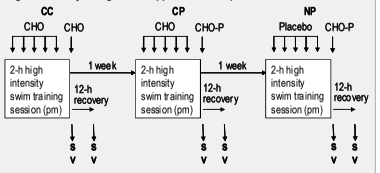
INTRODUCTION

- Type and timing of nutrient supplementation alter physiological response to exercise (1, 2).
- More research is necessary to determine a good supplementation protocol enhancing recovery from exercise in athletes (3).
- In this study, we have compared the acute effects of three supplementation protocols during and immediately after exercise on markers of muscle damage (plasma creatine kinase), stress (salivary cortisol), oxidative stress (protein carbonyls), and immune function (plasma and salivary cytokines IL-1b, IL-6, IL-8; salivary IgA) in elite swimmers.

METHODS

- Subjects (3 males and 6 females, age 18-24 y) were highly trained swimmers competing at national and/or international level.
- Design was a double-blind randomized cross-over trial of three supplementation protocols:
  - **CC**: carbohydrate beverage during and immediately after exercise.
  - **CP**: carbohydrate beverage during exercise, carbohydrate-protein mixture immediately after exercise.
  - **NP**: non-caloric water placebo during exercise, carbohydrate-protein mixture immediately after exercise.
- The time line of the study is shown in Figure 1.

Figure 1: Study design and supplementation protocols.



CHO, carbohydrate beverage; CHO-P, carbohydrate-protein beverage.
s, saliva sample; v, venous blood sample.

- Saliva and venous blood samples were taken immediately after exercise (pm) and 12 h later, before morning exercise (am).
- Before, during and after exercise athletes gave subjective ratings of coping with exercise, leg soreness and arm soreness.
- Markers were measured using commercially available kits.
- Analyses were performed using Excel spreadsheets (4). An effect was deemed unclear if its 90% confidence interval overlapped thresholds for substantiveness. Clear effects were assigned the observed magnitude, determined by standardization.

RESULTS

- See Figure 2.
- Athletes coped better with training in NP but the reduction in coping with training post NP may indicate recovery impairment.
- Soreness in legs and arms decreased in the mornings post all treatments. The reduction in leg soreness in CC was greater than in CP, because swimmers reported more pain in the legs before the start of CP.
- The reduction in plasma IL-6 and the increase in salivary IL-8 post CP contradict, as both cytokines are pro-inflammatory.
- The increase in protein carbonyl concentration post CC may be the result of more oxidative damage to protein.
- The increase in salivary IgA may indicate better immunity post CP.
- The reduction in morning cortisol with the CC treatment could be beneficial or harmful, depending on the role of cortisol at this time.

CONCLUSIONS

- Ratings of coping with training support carbohydrate ingestion during exercise for recovery.
- Inclusion of protein in a training supplement may reduce oxidative damage to protein.
- Consuming carbohydrate during exercise and carbohydrate-protein immediately after exercise increases salivary IgA and may therefore enhance immune function.
- More research is needed to determine a good supplementation protocol for athletes to use during their preparation for competition.

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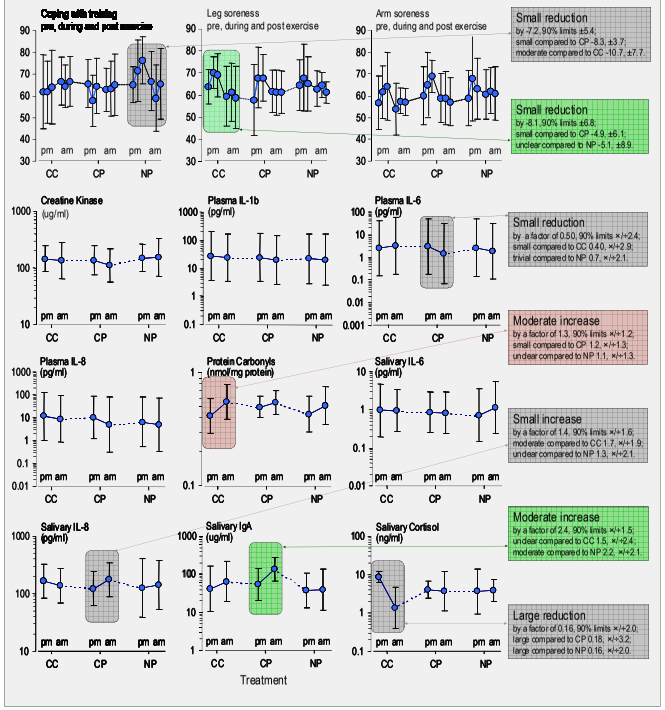
Figure 2: Subjective ratings and blood markers.

Clear effects are highlighted as follows: potentially harmful, potentially beneficial, uncertain role.
Data are means, bars are standard deviations.

Abbreviations for treatments:
CC: carbohydrate beverage during and immediately after exercise.
CP: carbohydrate beverage during exercise, carbohydrate-protein mixture immediately after exercise.
NP: non-caloric placebo during exercise, carbohydrate-protein mixture immediately after exercise.



APPENDIX B

Development and Validation of a Sensitive Immunoassay for the Skeletal Muscle Isoform of Creatine Kinase

Researchers at the Horticulture and Food Research Institute of New Zealand have developed an immunoassay specific for the skeletal muscle isoform of creatine kinase. They have used the blood samples taken for the study *Effects of Acute Dietary Interventions on Recovery from Training in Elite Swimmers*. My contribution to this study is mainly the provision of blood samples, and ensuring ethical approval for the study.

Lo, KR, Hurst SM, Atkinson KR, Vandenbogaerde T, Beaven, CM, Ingram, JR. 2008. Development and Validation of a Sensitive Immunoassay for the Skeletal Muscle Isoform of Creatine Kinase. Journal of Science and Medicine in Sport. 13:117-119.

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Original paper

Development and validation of a sensitive immunoassay for the skeletal muscle isoform of creatine kinase

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Abstract

Creatine kinase (CK) is a marker of muscle damage and pathology present as multiple tissue-specific circulating isoforms. CK is often measured using enzyme activity assays that are unable to distinguish these isoforms. We have developed an immunoassay specific for the MM isoform of CK, found predominantly in skeletal muscle, which uses very small volumes of plasma (1–2 μL). A sandwich enzyme-linked immunosorbent assay (ELISA) for CK-MM was developed using isoform-specific antibodies. Cross-reactivity with CK-BB and MB isoforms was also assessed. The ELISA was validated using plasma samples from a group of athletes, and the measured CK-MM concentrations were correlated with CK enzyme activity assays measured by a contractor using the same samples. The CK-MM ELISA has a limit of detection of 0.02 ng/mL, an IC_{50} of 2.3 ng/mL, and 5.8% cross-reactivity with CK-MB. CK-MM concentrations measured using this assay correlate well ($p < 0.0001$, Spearman $r = 0.89$) with enzyme activity assays. The CK-MM-specific ELISA can be used to help assess skeletal muscle damage independent of enzyme activity or interference from other CK isoforms, leading to more precise studies of muscle biology.

Keywords: Creatine kinase; Isoform; ELISA; Immunoassay; Muscle damage; Exercise

1. Introduction

Muscle damage can be diagnosed by measuring serum concentrations of biomarkers such as creatine kinase (CK). An increased concentration of this intracellular enzyme reflects cell damage and can occur after strenuous exercise or as a result of muscular pathologies[1]. Methods to quantify CK include kinase activity, immunoinhibition, immunofluorometric, and electrophoretic techniques[2,3], but these are not specific for the skeletal muscle form of CK. In addition, these techniques are influenced by changes in kinase activity such as those resulting from decreased extracellular glutathione[4].

CK is a dimeric protein formed from hetero- and homodimers of two subunits, B and M. Consequently, multiple isoenzymes occur, and each is found predominantly in a specific human tissue: MM in skeletal muscle, MB in heart, and BB in brain. CK-MB is a biomarker for heart trauma[5]. CK-MM is widely used as a skeletal muscle damage biomarker, especially in studies of exercise-induced muscle damage[6,7]. The presence of CK-MB can skew the results of assays assumed to measure CK-MM, especially after strenuous or prolonged exercise when CK-MB levels may also be increased[8]. Most current measurement techniques do not distinguish MM and MB isoforms. Previous CK-MM-specific immunoassays have exhibited relatively high (15%) cross-reactivity with other CK isoforms[9].

We have developed a sensitive immunoassay specific for the MM isoform of CK. Uniquely, this non-competitive sandwich ELISA requires very low sample volumes of 1–2 μL that are easily obtained using lancet methods. We have correlated the results of this immunoassay with CK enzymatic activity in plasma samples from a group of athletes.

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2. Methods

A monoclonal mouse anti-human CK-MM antibody #C7910-27 was obtained from US Biological (Swampscott, MA, USA). A polyclonal goat anti-human CK-MM antibody #70-XG47 and recombinant CK-MM #30-AC61, CK-MB #30-AC65, and CK-BB #30-AC56 proteins were obtained from Fitzgerald Industries International (Concord, MA, USA). SuperBlock® and 1-Step Ultra TMB-ELISA were obtained from Pierce Biotechnology (Rockford, IL, USA). All other materials were obtained from Sigma–Aldrich (Auckland, New Zealand).

This study was approved by the AUT University Ethics Committee (06/181) and written informed consent was obtained from all participants. All work conformed to the ethical guidelines of this committee. Nineteen healthy athletes, nine males and ten females, aged from 18 to 29 years (age 22 ± 3 years, weight 73 ± 9 kg, and height 180 ± 7 cm as mean ± S.D.), participated in the study. Six EDTA plasma specimens were collected from each subject by venipuncture, with one immediately following each of three 2 h high-intensity aerobic workouts and one 12 h after each workout. Specimen order and identification were randomised and all assays were performed blind.

For the ELISA, a 96-well plate was coated with 100 μL per well of monoclonal anti-human CK-MM antibody (diluted to 0.52 μg/mL with 0.05 M sodium bicarbonate pH 9.6) and incubated overnight at 4 °C. Non-specific binding wells were left uncoated. Wells were washed four times with phosphate-buffered saline plus 0.1% (v/v) Tween® 20 (PBST) using a microplate washer. The plate was then blocked for 1 h with 150 μL per well of a blocking solution consisting of 25% SuperBlock made up in assay buffer (0.1% bovine serum albumin, 0.1% ProClin® 300, 0.1% SuperBlock in PBST). The blocked plate was washed as described above. CK-MM standards were made at concentrations of 0, 0.01, 0.1, 1, 10, 100 and 1000 ng/mL. Standards, and serum samples diluted 100-fold with assay buffer were added (100 μL per well) and the plate was incubated at room temperature (RT) for 2 h. After washing, 100 μL per well of polyclonal anti-human CK-MM antibody, diluted to 0.33 μg/mL with assay buffer, was added and incubated at RT for 2 h. The plate was washed again before 100 μL per well of polyclonal anti-goat-HRP antibody, diluted 1:5000 (0.26 μg/mL) in assay buffer, was added. The plate was then incubated in the dark at RT for 45 min. After washing, 100 μL per well of substrate (1-Step Ultra TMB-ELISA) was added and mixed for 30 s. The plate was incubated for 5–10 min in the dark and the colour change was monitored visually. The reaction was stopped using 50 μL per well of 2 M H_2SO_4 and the plate was mixed for 30 s. Absorbance at 450 nm was read on a spectrophotometer. For ELISA cross-reactivity experiments, CK-BB and CK-MB standards were assayed at concentrations equal to those of CK-MM.

For enzymatic assays, plasma samples were diluted 1:1 in PBS and submitted to Medlab Hamilton Ltd, a commercial laboratory, where CK enzyme activity was determined using a MODULAR *ANALYTICS*<PP> analyzer (Roche Diagnostics, Basel, Switzerland). The assay measured the rate of formation of NADPH from creatine phosphate using standardised methods.

ELISA standards were fitted to the 5-parameter logistic (5PL) equation. Assay cross-reactivity and limit of detection were calculated using published methods[10]. Correlation between CK-MM ELISA and CK kinase activity was assessed with the Spearman test. Analyses were performed with GraphPad Prism V4.03 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

The standard curve of the CK-MM ELISA fits a 5PL curve with $r^2 = 0.99$. The assay detection limit was 0.02 ng/mL and the IC_{50} was 2.3 ng/mL with a dynamic range (IC_{20}, IC_{80}) of 0.5–4.6 ng/mL, corresponding to undiluted plasma concentrations of 51–463 ng/mL. Absorbances of non-specific binding controls and zero standards were well below the lowest CK-MM standard used (0.01 ng/mL). The cross-reactivity of this CK-MM ELISA with CK-MB was 5.8%. No cross-reactivity was observed with CK-BB. Inter- and intra-assay coefficients of variation for pooled plasma samples, respectively, were as follows: low (diluted concentration ~0.5 ng/mL), 10.7% and 6.6%; medium (~0.7 ng/mL), 6.5% and 8.0%; and high (~1.1 ng/mL), 8.8% and 7.2%.

During assay development, matrix effects were observed when assaying plasma. Two control plasma samples were serially diluted before assay, and alignment of the results for these two samples with the CK-MM standard curve showed that a minimum dilution factor of 1:20 is necessary for the present assay. We chose a dilution factor of 1:100 to enable use of a small sample volume and to limit matrix effects.

CK-MM concentrations were measured in 82 plasma samples collected from athletes immediately following a strenuous 2 h workout and after 12 h of recovery. CK enzymatic activity measurements were significantly correlated with CK-MM concentrations ($p < 0.0001$, Spearman $r = 0.89$).

4. Conclusions

This ELISA quantifies the MM isoform of CK with minimal cross-reactivity to other isoforms. Use of this ELISA provides a distinct advantage over enzyme activity assays, which cannot discriminate between the isoforms of CK found throughout the body. The results of this CK-MM ELISA correlate well with CK enzyme activity, but provide a more specific measurement of CK-MM release without influence from other isoforms or from solution conditions that may affect enzyme kinetics. The ELISA uses very small volumes of blood, such as those obtained easily by lancet,

enabling precise measurement of CK-MM concentrations from samples obtained using only minimally invasive techniques. While histological examination is needed to confirm damage to muscle fibers, the effects of exercise training and recovery techniques or other muscle damage protocols may be specifically assessed through use of this ELISA.

Acknowledgements

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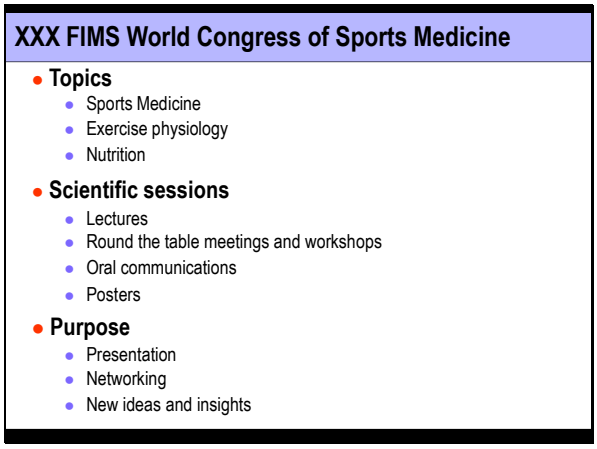
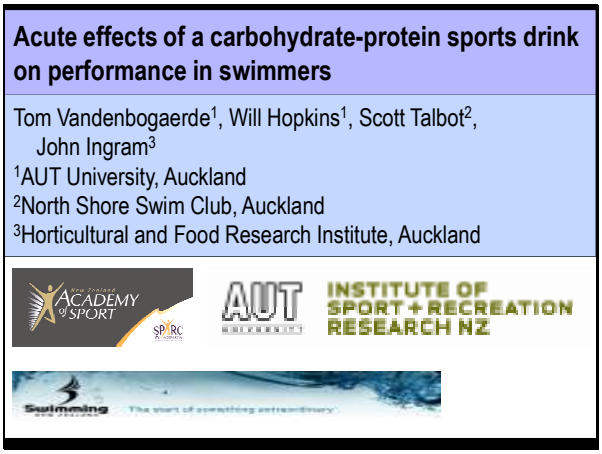
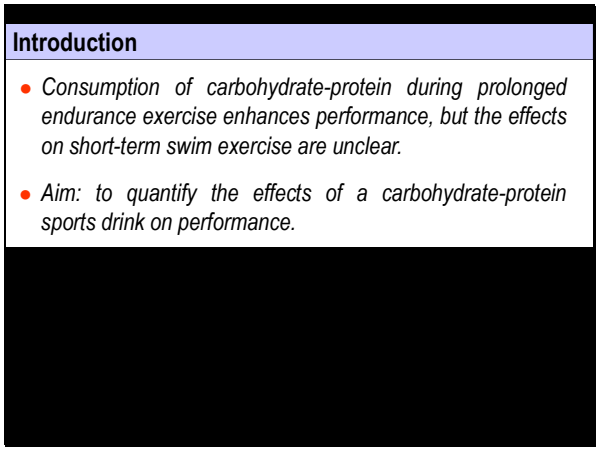
APPENDIX C

Powerpoint presentation for *Acute Effects of a Carbohydrate-Protein Sports Drink on Performance in Swimmers*

I gave this presentation at the XXX FIMS World Congress of Sports Medicine in Barcelona 2008.

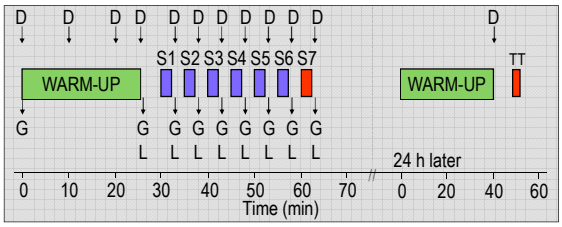






Methods

- *Study design*
  - Double-blind randomized crossover with a 1-wk wash-out.
  - 13 highly-trained swimmers consumed carbohydrate-protein or placebo before and during a 7x200-m step-test, and 10 min before a 100-m time-trial 24 h later.



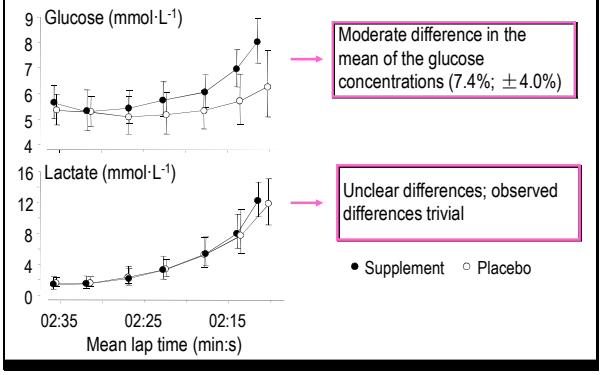
D, drink (supplement or placebo); S, step of step-test; G, glucose measurement; L, lactate measurement; TT, time-trial.

Results

- *Reliability of measurement*
  - Performance measures were highly reliable (1-wk standard errors of measurement were: Step 7, 1.6%; lactate threshold, 1.9%; 100-m time-trial, 0.8%).
- *Effects on performance*
  - Impairment in performance in Step 7 (0.9%; ±2.0%; possible harm, unlikely benefit);
  - Impairment in performance expressed as lactate threshold (0.2%; ±1.3%; possible harm, unlikely benefit);
  - Trivial effect on performance in the 100-m time-trial (0.1%; ±0.6%; unlikely harm, unlikely benefit).

Results

- *Glucose and lactate concentrations*



Discussion Points and Conclusion

- *Why did the drink impair performance in the step-test?*
  - Benefits of carbohydrate supplements on performance in short-term events and resistance exercise are not consistent.
  - High blood glucose might affect oxygen utilisation or net production of ATP, which may impair performance in short-term exercise.
- *Little effect on recovery from exercise?*
- *Conclusion*
  - The carbohydrate-protein supplement should not be used in short-term swim performance tests or races.
  - Because there were substantial effects on performance in the step-test, we suggest that researchers and coaches standardize nutrient supplementation protocols in such tests.

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- *Networking, new ideas and insights*
  - Didier Chollet => strength testing in swimming, use of lactates
  - Michael Gleeson => recovery from training and immunity
  - Luc Thomas => performance modeling
  - Filip Speybrouck => training programs cycling vs swimming

APPENDIX D

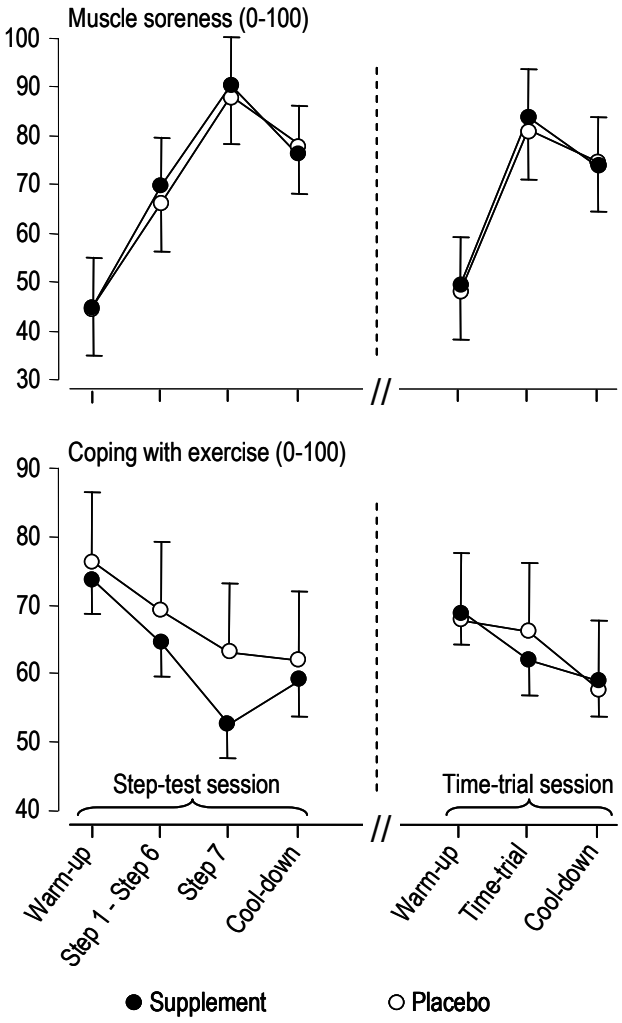
Subjective ratings of muscle soreness and coping with exercise in *Acute Effects of a Carbohydrate-Protein Supplement on Performance in Swimmers* (Chapter 2)

In *Acute Effects of a Carbohydrate-Protein Supplement on Performance in Swimmers*, the subjects rated muscle soreness and coping with exercise after the performance tests. We decided to remove these data from the paper because of our concern that the data would have been confounded by the knowledge of performance in the test.

TABLE. Subjective ratings in the step-test and time-trial sessions presented as differences between mean ratings (supplement minus placebo).

	Muscle soreness (0-100)	**Coping with exercise (0-100)**
Step-test		
Warm-up	0.0; ±7.1 trivial	-2.7; ±4.4 possible harm
Step 1 – Step 6	3.3; ±4.3 possible harm	-4.6; ±6.3 possible harm
Step 7	2.2; ±2.0 possible harm	-10; ±11 possible harm
Cool-down	-2.1; ±5.6 unclear	-3.1; ±8.5 trivial
Time trial		
Warm-up	1.2; ±3.5 trivial	1.5; ±4.8 unclear
Time trial	2.7; ±3.1 possible harm	-4.5; ±6.1 possible harm
Cool-down	-0.8; ±3.8 trivial	1.2; ±5.4 trivial

Data are differences in means with ±90% confidence limits and clinical inferences.



FIGURE—Subjective ratings of muscle soreness and coping with exercise with the supplement and placebo. Data are means and standard deviations.

APPENDIX E

Poster for *Monitoring Acute Effects on Athletic Performance with Mixed Linear Modeling*

This poster was presented by Will Hopkins on our behalf at the European College of Sports Science conference in Olso, 2009.

Monitoring acute effects on athletic performance with mixed linear modeling

Tom J. Vandenbogaerde and Will G. Hopkins

Institute of Sport and Recreation Research New Zealand, AUT University, Auckland, NZ

INTRODUCTION

- A sophisticated approach to quantify the small acute changes in performance with different conditions or treatments in elite athletes' training programs is lacking.
- We present mixed linear modeling as a novel and structured approach to monitor performance and to assess the effect of pharmacological or other interventions on performance in a squad of elite athletes whose performance is monitored regularly.

METHODS

- Subjects (6 males and 3 females, age 21-26 y) were highly trained swimmers competing at international level and specializing in 400-m freestyle (n=1), 100-m backstroke (n=1), 200-m backstroke (n=2), 100-m butterfly (n=1), 200-m butterfly (n=2), 100-m breaststroke (n=1) or 400-m individual medley (n=1).
- Each swimmer performed 2-8 time-trials in training and 2-7 in competition in the 9 wk prior to and including Olympic-qualifying trials; 0-4 time-trials were performed in the morning and 4-10 were performed in the afternoon.
- The athletes consumed caffeine of varying doses in some time-trials and a fixed dose (5mg·kg^{-1} body mass) or placebo in a double-blind, randomized, diet-controlled crossover manner in two training time-trials ≤2 wk apart.
- Data for one swimmer are shown in Figure 1.
- We used the mixed model procedure (Proc Mixed) in the Statistical Analysis System (Version 9.2, SAS Institute, Cary, NC) to quantify the changes in performance that occurred in training vs competition, in morning vs evening swims, and with use of caffeine and placebo.
- Predictor variables were coded as 0 or 1 to represent absence or presence of a condition and included in the model as fixed effects.
- Date of each performance test was included as a continuous linear fixed effect and interacted with the random effect for athlete to represent individual differences in linear trends in performance.
  - When data show nonlinear trends, it would be a simple matter to model the trends as polynomials.
  - More complex curvature requires nonlinear mixed modeling, a challenging procedure in SAS.
- To investigate the effect of caffeine on longer vs shorter swims, duration of the time-trial was included in the model as a log-transformed value interacted with caffeine (present or absent) and number of caffeine capsules. The effect of time-trial duration on the effect of caffeine dose was estimated as the change in the effect of caffeine for a doubling of time-trial time.
- Effects were estimated as percentage improvements or impairments in performance.
- We used clinical inferences: an effect with possible benefit (>25% chance) was clear if harm was very unlikely (odds ratio of benefit/harm >66) and unclear otherwise; other effects were clearly not beneficial.

RESULTS

- Performance was highly reliable in time-trials in training and in competition (typical errors both 0.8%).
- Performance time improved linearly by 0.8% (90% confidence interval 0.3% to 1.3%) per 4 wk of training, with individual differences (standard deviation) in the trend of 0.5% (0.3 to 1.2%) per 4 wk.
- The swimmers performed better in evenings vs mornings by 0.6% (0.1 to 1.1%) and in competition vs training by 1.4% (0.9 to 1.9%).
- A 100-mg dose of caffeine enhanced performance in training time-trials by 1.3% (-0.1 to 2.4%) and in competition time-trials by 1.5% (0.3 to 2.5%); each additional 100 mg reduced the benefit slightly by an unclear 0.1% (-0.3 to 0.5%).
- The effect of a doubling of time-trial time on the effect of caffeine was an unclear -0.0% (-1.6 to 1.5%).
- The placebo effect was a slight but unclear impairment of 0.2% (-0.6 to 0.9%).

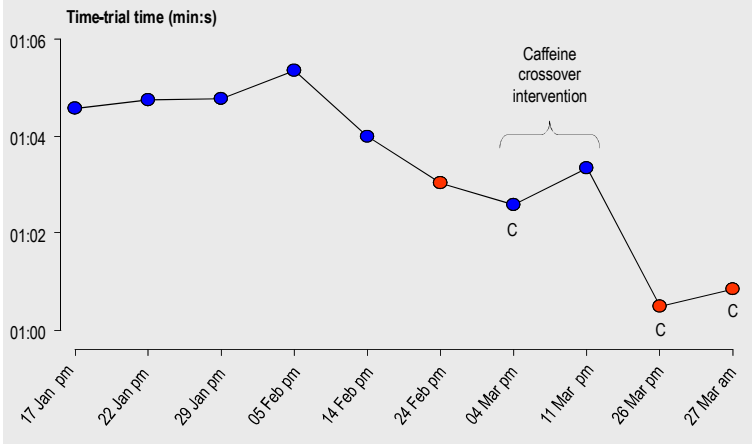
CONCLUSIONS

- We successfully used mixed linear modeling of elite swimmers' performance times to demonstrate and quantify:
  - individual trends in performance with training;
  - better performance in evenings vs mornings;
  - better performance in competitions vs training time-trials;
  - better performance with caffeine.

ABSTRACT

There is a need for a sophisticated approach to track athletic performance and to quantify factors affecting it in practical settings. **Purpose:** To demonstrate the utility of mixed linear modeling for monitoring athletic performance. **Methods:** Elite swimmers (3 females and 6 males; age 21-26 y) performed 2-8 time-trials in training and 2-7 in competition in the 9 wk prior to and including Olympic-qualifying trials, all in the stroke and distance of the athlete's main event. We included a double-blind, randomized, diet-controlled crossover intervention in which the swimmers consumed caffeine (5 mg·kg^{-1} body mass) or placebo 75 min before two training trials ≤2 wk apart. The swimmers also knowingly consumed varying doses of caffeine in some other trials and in competitive swims. We used mixed linear modeling of log-transformed swim time to quantify performance in training vs competition, in morning vs evening swims, and with caffeine vs placebo. Predictor variables were coded as 0 or 1 to represent absence or presence of each condition and included as fixed effects. Date of each performance test was included as a continuous linear fixed effect and interacted with the random effect for athlete to represent individual differences in linear trends in performance. Outcomes were deemed unclear if the 90% confidence interval included substantial enhancement and impairment (0.3%). **Results:** Performance times in the time-trials and competitions were highly reliable (typical errors both 0.8%). Performance time improved linearly by 0.8% (90% confidence interval 0.3% to 1.3%) per 4 wk of training, with individual differences (standard deviation) in the trend of 0.5% (0.3 to 1.2%) per 4 wk. The swimmers performed better in evenings vs mornings by 0.6% (0.1 to 1.1%) and in competition vs training by 1.4% (0.9 to 1.9%). A 100-mg dose of caffeine enhanced performance in time trials by 1.3% (-0.1 to 2.4%) and in competitions by 1.5% (0.3 to 2.5%); each additional 100 mg reduced the benefit slightly by an unclear 0.1% (-0.3 to 0.5%), and the placebo effect was also a slight but unclear impairment of 0.2% (-0.6 to 0.9%). **Conclusion:** Mixed linear modeling is a successful approach for quantifying small changes in performance in a squad of elite athletes whose performance is monitored regularly over a period of weeks-months.

Figure 1: Time-trials in 100-m breaststroke performed in training (●) and competition (●) in one elite swimmer. C indicates use of caffeine in the corresponding time-trial.



Swimming New Zealand — The start of something extraordinary

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APPENDIX F

Scales used in Study 1 and Study 3

How do you rate last night's sleep overall?

100	perfect sleep
95	very very good sleep
90	very very good sleep
85	very good sleep
80	very good sleep
75	very good sleep
70	good sleep
65	good sleep
60	good sleep
55	average sleep
50	average sleep
45	average sleep
40	poor sleep
35	poor sleep
30	poor sleep
25	very poor sleep
20	very poor sleep
15	very poor sleep
10	very very poor sleep
5	very very poor sleep
0	no sleep

Ritchie SE, Hopkins WG. The intensity of exercise in deep-water running. Int J Sports Med 12: 27-29, 1991.

How do you rate your focus before the race?

100	perfect focus
95	very very good focus
90	very very good focus
85	very good focus
80	very good focus
75	very good focus
70	good focus
65	good focus
60	good focus
55	moderate focus
50	moderate focus
45	moderate focus
40	poor focus
35	poor focus
30	poor focus
25	very poor focus
20	very poor focus
15	very poor focus
10	very very poor focus
5	very very poor focus
0	no focus

Ritchie SE, Hopkins WG. The intensity of exercise in deep-water running. Int J Sports Med 12: 27-29, 1991.

How much are you aching or hurting?

100	unbearable
95	very very much
90	very very much
85	very much
80	very much
75	very much
70	quite a lot
65	quite a lot
60	quite a lot
55	some
50	some
45	some
40	a little
35	a little
30	a little
25	very little
20	very little
15	very little
10	very very little
5	very very little
0	no pain at all

How well did you cope?

100	perfectly
95	very very well
90	very very well
85	very well
80	very well
75	very well
70	well
65	well
60	well
55	moderately
50	moderately
45	moderately
40	poorly
35	poorly
30	poorly
25	very poorly
20	very poorly
15	very poorly
10	very very poorly
5	very very poorly
0	terribly

Ritchie SE, Hopkins WG. The intensity of exercise in deep-water running. Int J Sports Med 12: 27-29, 1991.

APPENDIX G

A Competition-Based New Research Design to Assess an Intervention Affecting Performance of a Squad of Elite Athletes

Vandenbogaerde, T.J.,[1] Hopkins, W.G.[1] and Pyne, D.B.[2]

[1]Auckland University of Technology, New Zealand; [2]Australian Institute of Sport, Australia

I will present this study at the XIth International Symposium on Biomechanics and Medicine in Swimming in Oslo 2010.

INTRODUCTION

Performance of elite athletes is difficult to study with conventional experimental designs, because sample sizes are usually small, coaches are often unwilling to randomize athletes to treatments, and performance in tests may not reflect performance in competitions. We present here a more practical powerful competition-based research design for sports in which a squad of athletes competes frequently as individuals and the coach is prepared to implement an intervention for a competition.

METHODS

We developed and evaluated the competition-based design by analyzing US competitive swimming performance times, assuming an intervention affecting performance in all strokes and distances had been applied to swimmers in one or more arbitrarily chosen squads for one or more competitions. The swim times were downloaded from USAswimming.org for the period September 2008 through August 2009 (post Beijing Olympics through Rome World Championships and US Open Championships). We analyzed data for swimmers who achieved >900 FINA points at the World Championship selection trials and who were in squads of >2 swimmers at the trials. Each swimmer's best points score was then used to select their best event, and only competitions with >14 best-event swims were included. Times for the resulting 363 swims in 7 competitions by 146 athletes in 19 squads were then analyzed with a mixed linear model. Fixed and random effects were included in the model to estimate the intervention effect in a design equivalent to a parallel-groups controlled trial.

RESULTS

Uncertainty in the estimate of the mean effect of an intervention in a given competition with a squad of ~13 swimmers was a minimum of ±0.8% (expressed as 90% confidence limits) when there were at least 50 other swimmers in that competition and when all swimmers entered at least four other competitions. The same intervention applied to an extra squad (~25 swimmers in total) in a different competition reduced the minimum uncertainty to ±0.5%.

DISCUSSION

The competition-based research design appears to provide outcomes that are more precise and trustworthy than those of usual research designs for interventions on athletic performance, but clear outcomes with interventions producing trivial or smallest important effects for swimmers (~0.3%[1,2]) will require use of multiple squads in multiple competitions.

REFERENCES

1. Pyne, D.B. et al., J Sports Sci 22, 613-620, 2004
2. Hopkins, W.G. et al., Med Sci Sports Exerc 41, 3-12, 2009