An evidence-based approach to the application of the science of sports and exercise nutrition to optimising sporting performance

Lukas Y. Beis, BSc (Hons)

Submitted in fulfilment of the requirements for the

Degree of Doctor of Philosophy

College of Medicine, Veterinary & Life Sciences

Institute of Cardiovascular & Medical Sciences

University of Glasgow

February 2012

Abstract

The primary objective of this series of experiments was to explore some of the reasons which lead to equivocal outcomes in the literature on dietary recommendations and to demonstrate the need for evidence-based data on well-trained and elite athletes. Therefore, the previous mentioned groups of athletes were recruited to participate in four research studies, each entailing a series of experimental trials. The data obtained were compared to previous research and/or to the established dietary recommendations.

The aim of the first research study presented in this thesis (Chapter 2) was to examine the effects of Glycine-arginine-α-ketoisocaproic acid (GAKIC) supplementation on fatigue during high intensity, repeated cycle sprints in trained cyclists. It should be noted that despite the fact that studies on GAKIC supplementation involving well-trained subjects are lacking, athletes regularly use this commercially available supplement. This is is also the case for a vast range of other supplements whose suitability has been assessed in normal healthy subjects or recreationally active individuals. In the study presented in Chapter 2, 10 well-trained male cyclists completed two supra-maximal sprint tests each involving 10 sprints of 10 s separated by 50 s rest intervals on an electrically braked cycle ergometer. Participants ingested 11.2 g of GAKIC (according to protocols previously established in the literature) or Placebo (Pl) during a period of 45 min prior to the experimental trials. Peak power, mean power, fatigue index as well as heart rate (HR) and ratings of perceived exertion did not differ between conditions (GAKIC vs. Pl). Peak power declined from the 1st sprint (mean ± SD) (Pl: 1332 ± 307 W, GAKIC: 1367 ± 342 W) to the 10th sprint (Pl: 1091 ± 229 W, GAKIC: 1061 ± 272 W) and did not differ between conditions (P = 0.88). Mean power declined from the 1st sprint (Pl: 892 ± 151 W, GAKIC: 892 ± 153 W) to the 10th sprint (Pl: 766 ± 120 W, GAKIC: 752 ± 138 W) and did not differ between conditions (P = 0.96). Fatigue index remained at ~38% throughout the series of sprints and did not differ between conditions (P = 0.99). HR and ratings of perceived exertion increased from the 1st sprint to the 10th sprint and did not differ between conditions (P = 0.11 and P = 0.83, respectively). The data reported, suggest that GAKIC has no ergogenic effect on repeated bouts of high intensity exercise in trained individuals. The reported data further contradicts previous performance studies where GAKIC was found to attenuate the decline in power output, improve muscle performance and delay muscle fatigue resulting in the improvement of total work during high intensity exercise. Notably, none of the previous studies involving GAKIC supplementation seem to control for a number of possible

confounding factors that could have adversely affected the results. For instance, utilization of untrained individuals and failure to include baseline trials to establish the repeatability of performance trials leaves the reliability of the data open to question. Furthermore, the results in Chapter 2 highlight the importance of extrapolating decisions concerning the effectiveness of a marketed nutritional supplement from the best available research conducted on well-trained subjects.

The aim of Chapter 3 was to assess the food and macronutrient intake of elite Ethiopian distance runners; a group of athletes that dominates endurance running. The results of the research allowed further examination and direct comparison of the nutrient intake to the established guidelines and previous studies conducted on African runners. The dietary intake of 10 highly-trained Ethiopian long distance runners, living and training at high altitude was assessed during a 7-day period of intense training prior to competition using the standard weighed intake method. Training was also assessed using an activity/training diary. Body mass (BM) was stable (i.e., was well maintained) over the assessment period (pre: 56.7 ± 4.3 kg vs. post: 56.6 ± 4.2 kg, $P = 0.54$). The diet comprised of 13375 ± 1378 kJ and was high in carbohydrate (64.3 ± 2.6% of total energy intake (TEI), 545 ± 49 g, 9.7 ± 0.9 $g \cdot kg^{-1}$). Fat and protein intake was 23.3 ± 2.1% TEI (83 ± 14 g) and 12.4 ± 0.6% TEI (99 ± 13 g, 1.8 ± 0.2 $g \cdot kg^{-1}$), respectively. Fluid intake comprised mainly of water (1751 ± 583 $mL \cdot day^{-1}$), while no fluids were consumed before or during training with only modest amounts being consumed following training. It was concluded that, as found in previous studies on elite Kenyan distance runners, the diet of these elite Ethiopian distance runners met most recommendations for endurance athletes in regard to macronutrient intake but not in regard to fluid intake. Nevertheless, it remains unclear in what way these differences in fluid consumption, before major competitions, have an impact on their performance. Therefore, Chapter 3 highlights the fact that more studies involving truly world-class athletes are required in order to assess and possibly improve the applicability of current recommendations to elite athletes. Chapter 3 also highlights the potential role of the commercial industry in the application of the science of exercise nutrition.

Chapter 4 aimed to describe the drinking behaviours of elite male marathon runners during major city marathons. Retrospective video analysis of 10 male marathon runners during 13 major city marathons was undertaken. Total drinking durations during the marathons were determined by estimating the time spent ingesting fluid at each drinking station from video images. The ambient conditions during the 13 studied marathon races were 15.3 ± 8.6 ^{o}C

(ambient temperature) and 59 ± 17% relative humidity; average marathon competition time was 02:06:31 ± 00:01:08 (h:min:s). Total drinking duration during these races was 25.5 ± 15.0 s (range: 1.6 - 50.7 s) equating to an extrapolated fluid intake rate of 0.55 ± 0.34 $L \cdot h^{-1}$ (range: 0.03 - 1.09 $L \cdot h^{-1}$). No significant correlations were found between total drink duration, fluid intake (rate and total), running speed and ambient temperature. Estimated BM loss based on calculated sweat rates and rates of fluid ingestion was 8.8 ± 2.1% (range: 6.6 - 11.7%). Measurements of the winner in the 2009 Dubai marathon revealed a BM loss of -9.8%. It was concluded that the most successful runners during major city marathons, drink fluids *ad libitum* (i.e., at one's pleasure) for less than ~60 s at an extrapolated fluid ingestion rate and is in accordance with the current recommendations by the American College of Sports Medicine of 0.4 - 0.8 $L \cdot h^{-1}$. Nevertheless, these elite runners do not seem to maintain their BM within the current recommended ranges of 2 – 3%. On the other hand, this apparently widely adopted *ad libitum* strategy during marathon racing seems to produce optimal/winning performances. This evidence and the finding that the runner, who set the previous world record (2008), finished a competitive race (Dubai, 2009) with a BM loss of 9.8%, suggest that a tolerable range for dehydration may exist. It is possible, that this tolerable limit of dehydration may not have a negative impact on running performance in elite runners and may even confer an advantage by preventing a significant increase in BM due to "over - consumption" of large volumes of fluid.

Given the data extrapolated from "real world" studies (Chapters 3 and 4) and the established guidelines for fluid ingestion, the investigation in Chapter 5 aimed to examine a possible method that could bring together the established guidelines and the data extracted from "real world" studies. Therefore, the effects of a hyper-hydration method combining creatine (Cr) and glycerol (Gly) supplementation on thermoregulatory responses and running economy (RE) in hot and cool conditions were investigated. $Cr \cdot H_2O$ (11.4 g), Gly (1 $g \cdot kg^{-1}$ BM) and glucose polymer (75 g) were administered twice daily to 15 male endurance runners during a 7-day period. Exercise trials were conducted pre- and post-supplementation at 10 and 35 °C and 70% relative humidity. Combined Cr and Gly supplementation increased BM and total body water by 0.90 kg and 0.71 L, respectively following supplementation. Despite the significant increase in BM, supplementation had no effect on oxygen uptake ($\dot{V}O_2$) and thus RE. Both HR and core temperature were attenuated significantly after supplementation. Combining Cr and Gly is effective in reducing thermal and cardiovascular strain during exercise in the heat without negatively impacting RE.

The potential influence of the commercial industry on scientific objectivity, as well as the lack of properly evaluated, controlled and randomized studies are the two main weaknesses that prevent the establishment of well accepted guidelines for food and fluid intake of well-trained and elite athletes. The development of novel guidelines needs to be solely evidence-based. Therefore, in order to reach conclusions regarding specific categories of athletes, research should be conducted on homogeneous groups (i.e., either well-trained, or elite, or world-class). Furthermore, research must be conducted under environmental and other conditions that are equivalent to those met during ‘‘outdoor’’ exercise, in order to evaluate and even improve the prevailing recommendations.

Table of contents

List of Tables

List of Figures

Acknowledgments

Four years ago, full of dreams and concerns, I decided to follow a very important and difficult path. The interesting part of my path was that due to financial and time related issues, I firstly applied for an MSc. However, my supervisor, Dr Yannis Pitsiladis, inspired me to extend my studies. Dr Yannis Pitsiladis, therefore, provided me with the opportunity to become involved with in depth novel research studies, which formulated this PhD thesis. Indeed, visiting Ethiopia, Dubai, USA, England, Portugal, Holland, Cyprus to conduct research and also to present work at the highest standard of international meetings is not a usual path for a PhD student. I would like to express my gratitude to my supervisor, Dr Yannis Pitsiladis, and the University of Glasgow for providing me with this opportunity. To my supervisor: Your scientific approach and your enthusiasm for work will always be an inspirational example to follow.

This thesis would not have been possible without considerable help and support from a number of individuals, participants, collaborators, friends and family.

Massive thanks to the PhD students and Post docs in the University for making our labs and office great places to study and work in the last few years. In particular, I thank Thelma, Christina, Maria, Carlos, Laura, Hanna, Michael, Robert O, Yaser, Guan, as well as Chris and Robert S. Lunches, cups of tea and coffees, dinners, nights out, conference trips made the bad times seem good and the good times even better.

Special mentions to Ramzy and Lena for the unforgettable work trip to Ethiopia in 2008.

In between lab work, studying and writing there was time for enjoying the life in Glasgow. In particular, I thank Kostas, Eirini, Asrar, Omar and the aforementioned people for the beautiful times spent in Glasgow.

Many thanks to John Wilson, Ian Watt and Anne Keenan. Without their technical assistance throughout my experimental studies, my research experiments would have not run as smoothly.

I would like to thank the undergraduate students who have helped in the experimental trials over the years.

A thank you to the subjects who have performed in all the experimental trials mentioned in this thesis. To put yourself through hell in such an enthusiastic manner all in the name of science is truly inspirational.

I am very thankful to Thelma Polyviou, Barry Fudge and Panagiota Papaioannou who supported me with their time and experience, and provided me with comments on my PhD thesis.

A special thank to Fanis Kollias for giving me the opportunity to make some money throughout the last year of my PhD.

Special thanks to Kostas and Petros for spending a fortune on phone calls and keeping in close touch over the PhD years as well as Yiorgos and Aleksis for travelling to Glasgow to visit me.

An enormous thank you to Vassiliki Athanasiou for all the "skype- marathons". You have been through it all with me. You have supported me with all your love and patience.

To my brother Leonidas and my sister Vassiliki for always being there and for their constant love and encouragement.

Last but not least, I would like to thank my mum and dad. Without their support throughout the years I would not be where I am today. To my parents: You made me believe in my skills and you supported me financially and mentally, provided motivation, and lifted me up when I was down and words cannot express how much all this has meant to me. I know I will never be able to repay you in full but I hope I have gone some way in doing so.

Author's declaration

I hereby declare that this thesis has been composed by myself, and that the work of which it is a record has been done by myself, except where specifically acknowledged. I also confirm that it has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of references.

Lukas Y. Beis _____ Date_____

Some of the results contained in this thesis have been published in peer-reviewed journals as follows:

1. **Lukas Beis**, Yaser Mohammad, Chris Easton, and Yannis P. Pitsiladis (2011). Failure of glycine-arginine-α-ketoisocaproic acid to improve high intensity exercise performance. International Journal of Sport Nutrition and Exercise Metabolism 21(1): 33-39

2. **Lukas Y Beis**, Lena Willkomm, Ramzy Ross, Zeru Bekele, Bezabhe Wolde, Barry Fudge, and Yannis P Pitsiladis (2011). Journal of the International Society of Sports Nutrition 8: 7

3. **Lukas Beis**, Moray Wright-Whyte, Barry Fudge, Tim Noakes, and Yannis P. Pitsiladis (2012). Drinking behaviours of elite male runners during marathon competition. Clinical Journal of Sport Medicine. (In press)

4. **Lukas Y. Beis**, Thelma Polyviou, Dalia Malkova, and Yannis P. Pitsiladis (2011). The effects of hyperhydration on running economy in well trained endurance runners. Journal of the International Society of Sports Nutrition. 16; 8(1): 24. [Epub ahead of print].

Some of the results contained in this thesis have been presented at conferences as follows:

1. **Lukas Beis**, Yaser Mohammad, Hannah Budd, Lena Willkomm, David Kingsmore, Lesley Hall, John Wilson, Heather Collin, Chris Easton, Yannis P. Pitsiladis (2008). Glycine-arginine-alpha-ketoisocaproic acid does not improve performance of

repeated supra-maximal cycling sprints in trained cyclists. 13th Annual Congress of the European College of Sport Science. "Sports Science by the sea" Estoril-Portugal 9-12 July 2008.

2. **Lukas Y. Beis**, Lena Willkomm, Ramzy Ross, Zeru Bekele, Bezabhe Wolde , Yannis P. Pitsiladis (2009). Assessment of dietary intake of elite Ethiopian distance runners. American College of Sports Medicine (ACSM), 56th Annual Meeting. Seattle, Washington, 27 – 30 May 2009.

3. **Lukas Beis**, Moray Wright-Whyte, Barry Fudge, Tim Noakes, and Yannis P. Pitsiladis (2010). Drinking behaviours of elite male runners during marathon competition. British Association of Sport and Exercise Science (BASES) Conference "Challenging the Dogma" Glasgow, 6-8 September 2010.

Some of the results contained in this thesis have been accepted for publication in encyclopedia book chapters as follows:

1. Lukas Beis, Yannis Pitsiladis (invited contribution) (2012). Hydration assessment during long distance running. Encyclopedia of Exercise Medicine in Health and Disease. Springer (UK). Edited by Mooren, Frank-Christoph.

Some of the experiments presented in this thesis utilise a composition protected by the following patent:

1. Novel Hyperhydration solution (submitted by the University of Glasgow in Great Britain and USA, 23rd July 2004). Patent/GB2005/002913 - Hydrating Composition

Dedication

When you set out on your journey to Ithaca,
pray that the road is long,
full of adventure, full of knowledge.
The Lestrygonians and the Cyclops,
the angry Poseidon - do not fear them:
You will never find such as these on your path,
if your thoughts remain lofty, if a fine
emotion touches your spirit and your body.
The Lestrygonians and the Cyclops,
the fierce Poseidon you will never encounter,
if you do not carry them within your soul,
if your soul does not set them up before you.

Pray that the road is long.
That the summer mornings are many, when,
with such pleasure, with such joy
you will enter ports seen for the first time;
stop at Phoenician markets,
and purchase fine merchandise,
mother-of-pearl and coral, amber and ebony,
and sensual perfumes of all kinds,
as many sensual perfumes as you can;
visit many Egyptian cities,
to learn and learn from scholars.

Always keep Ithaca in your mind.
To arrive there is your ultimate goal.
But do not hurry the voyage at all.
It is better to let it last for many years;
and to anchor at the island when you are old,
rich with all you have gained on the way,
not expecting that Ithaca will offer you riches.

Ithaca has given you the beautiful voyage.
Without her you would have never set out on the road.
She has nothing more to give you.

And if you find her poor, Ithaca has not deceived you.
Wise as you have become, with so much experience,
you must already have understood what Ithacas mean.

(Cavafy, 1911)

Although the English version seems to lack the melodic pace that characterises the original Greek version, I still find it amazing and I have always used it as a guiding compass for my PhD, my education years, and for my entire life.

Definitions/Abbreviations

ACSM	American College of Sports Medicine
AGAT	Arginine: Glycine amino-transferase
ANOVA	Analysis of variance
BM	Body Mass
BMR	Basal Metabolic Rate
CHO	Carbohydrate
Cr	Creatine
C.V.	Coefficient of Variation
ECW	Extracellular water
EE	Energy Expenditure
EI	Energy Intake
FDA	Food and Drug Administration
DSHEA	Dietary Supplement Health and Education Act
GAKIC	Glycine-arginine-α-ketoisocaproic acid
Gly	Glycerol
GSSI	Gatorade Sports Science Institute
H	Hour
Hct	Haematocrite
Hb	Haemoglobin
HR	Heart Rate
IAAF	International Association of Athletic Federations
ICW	Intracellular water
IMMDA	International Marathon Medical Directors Association
KIC	-α-ketoisocaproic acid
PAR	Physical Activity Ratio
PCV	Packed - cell volume
Pl	Placebo
PV	Plasma Volume
RER	Respiratory exchange ratio
RE	Running Economy
RPE	Rating of Perceived Exertion
SD	Standard Deviation
TBW	Total Body Water

TC	Thermal Comfort
T_{core}	Core Temperature
TEI	Total Energy Intake
$\dot{V}CO_2$	Carbon Dioxide production
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_2max$	Maximal Oxygen Uptake
WADA	World Anti-Doping Agency

1. General Introduction

1.1 Factors affecting exercise performance

Elite athletes (i.e., athletes with potential for competing in the Olympics) and world-class athletes (i.e., athletes who are amongst the best in the world) are constantly looking for "the edge" to optimise exercise performance in order to reach their maximal performance level. In fact, the international standard in sports is typically on an upward trend. As a result athletes constantly endeavour to find new strategies in order to maintain and/or improve their competitive edge. Therefore, training and competition preparation for athletes should focus on the improvement of factors, which have in the past shown to positively affect exercise performance. Talent is undoubtedly the most important attribute of the elite performer, but it is difficult to define (Maughan, King, & Lea, 2004). Although there are a variety of factors essential to world-class exercise performance, the principle of optimal training and recovery appears to be an inviolable rule for optimal performance (Myburgh, 2003). According to Myburgh (2003), the formation of research on performance contributors can be represented by a pyramid model (Figure 1.1) where optimal training and recovery are always found at the base of this multidisciplinary approach (Figure 1.1). Myburgh's model (2003) proposes that conventional methods (i.e., physiology, biochemistry and histology) may be insufficient for assessing and distinguishing between elite and truly world-class athletes. Hence, a multidisciplinary approach that also includes molecular biology and genetics must be embraced. The study by Williams and Folland (2008) is also in agreement with the aforementioned evidence. These authors (Williams & Folland, 2008) mention that human physical performance is multi-factorial and is determined by a range of genetic and environmental factors (physical training, nutrition and technological aids). When viewing Olympic and world records in athletic events as indicators of the limits of human physical potential, it should be recognised that a performance record is a function of economic and social opportunities in addition to genetic potential and physical environment (i.e., nutrition, economics and culture) (Williams & Folland, 2008).

1.1.1 Genetics factors affecting performance

A defining factor affecting exercise performance is genetics (Hamilton, 2000; Scott et al., 2009). Undoubtedly, there are many interacting genes involved in exercise-related traits,

including sporting performance (Pitsiladis & Wang, 2011). Nevertheless, there has been limited progress in determining genetic contribution to fitness phenotypes due to few coordinated research efforts and the use primarily of the candidate gene approach (Pitsiladis & Wang, 2011). For example, despite a growing number of candidate genes, only two nuclear candidate genes have been investigated in elite Kenyan runners, the ACE and ACTN3 genes (Scott et al., 2005; Yang et al., 2007). It should be noted that East African middle and long distance runners are currently the dominant force in athletics. Notably, Scott et al. (Scott et al., 2009) compared DNA samples obtained from Kenyan athletes who compete either on a national or international level with samples obtained from the general Kenyan population. The authors (Scott et al., 2009) found that the international level athletes showed a significantly different distribution of mtDNA haplogroups relative to control subjects (general Kenyan population) and national standard athletes. The implication however, is that there is undiscovered potential for a further understanding of the capacity of endurance by researching the human genome extensively (Klissouras, 1971; Pitsiladis & Wang, 2011). The genetic factor is also related to training (since realisation of genetic potential requires interaction with environmental stimuli, which in the case of athletic performance is training) (Myburgh, 2003). This may also be corroborated by the aforementioned investigation (Williams & Folland, 2008), which attributes the success of East African endurance runners as likely to be the interplay of complex genetic factors and their surrounding environment (Williams & Folland, 2008). Without the interaction between each genetic element and the environment, world-class performance will never be achieved (Myburgh, 2003; Williams & Folland, 2008). Hence, the model of Myburgh et al. (2003) does not make genetic analysis the basis of elite endurance performance, as this would not be sufficient in assessing and distinguishing between elite and truly world-class athletes. A multidisciplinary approach is necessary in order to obtain a clear understanding of the performance of athletes and the examination of other factors including biochemistry, molecular biology and physiology, must also be considered (Myburgh, 2003; Williams & Folland, 2008).

1.1.2 Physiological factors affecting exercise performance

To become an elite and/or world class athlete, it is necessary to have the correct physiology since there are key physiological factors which seem to be fundamental in order to win at the highest level. For example, elite endurance performance is a complex, multi-factorial phenotype characterized by several physiological adaptations. Firstly, high maximal oxygen uptake values ($\dot{V}O_2max$), defined as the highest rate at which oxygen can be taken

up and utilized by the body during severe exercise, are undoubtedly necessary for successful performance in elite endurance running (Bassett & Howley, 2000; Sjodin & Svedenhag, 1985). For instance, to complete a 02:15:00 (h:min:s) marathon, a $\dot{V}O_2$max of about 60 $mL \cdot kg^{-1} \cdot min^{-1}$ must be maintained throughout the run. Since the marathon is typically ran at about 80–85% of $\dot{V}O_2$max, the $\dot{V}O_2$max values needed for that performance would be 70.5-75 $mL \cdot kg^{-1} \cdot min^{-1}$ (Bassett & Howley, 2000). Furthermore, running economy (RE) (i.e., the sub-maximal $\dot{V}O_2$ at a given running velocity) is also a major contributor for successful running (Bassett & Howley, 2000; Saunders, Pyne, Telford, & Hawley, 2004b). Di Prampero et al. (1993) stated that a 5% increase in RE induced an approximately 3.8% increase in distance running performance (Di Prampero et al., 1993). Early research comparing elite runners with $\dot{V}O_2$max values at 79 $mL \cdot kg^{-1} \cdot min^{-1}$ with good distance runners ($\dot{V}O_2$max values at 69.2 $mL \cdot kg^{-1} \cdot min^{-1}$) indicated that elite runners had better RE than good runners. When expressed as a percentage of $\dot{V}O_2$max, this difference in RE was magnified, with the elite runners working at a lower percentage of their $\dot{V}O_2$max (Saunders, Pyne, Telford, & Hawley, 2004). The RE of athletes with similar $\dot{V}O_2$max values can vary up to 30% (Daniels, 1985). The relationship between RE and performance is well documented. However, data on the extent to which RE can improve with continued training are limited to a few case reports that have followed athletes over a number of years. A study by Jones (Jones, 2006) reported a 15% improvement in RE in a longitudinal (over 7 years) laboratory physiological study conducted on Paula Radcliffe (the current women's marathon world record holder), while no changes were observed in $\dot{V}O_2$max during the same period. The study by Jones is also in agreement with the data extracted from Lance Armstrong (seven times Tour de France winner) (Coyle, 2005) whose muscular efficiency when cycling improved by 8% over a 7 year period. A case study of the American mile record holder Steve Scott (Conley, Krahenbuhl, Burkett, & Millar, 1984) is a further example of the relationship between RE and performance. Conley, et al. (1984) reported that during a 6-month period of training, Scott improved his $\dot{V}O_2$max by 3.8% (74.4 to 77.2 $mL \cdot kg^{-1} \cdot min^{-1}$) with a greater improvement of 6.6% in RE (48.5 to 45.3 $mL \cdot kg^{-1} \cdot min^{-1}$) at a running velocity of 16 $km \cdot h^{-1}$. The combined improvement of an increased $\dot{V}O_2$max and a better RE reduced the relative intensity of running at 16 $km \cdot h^{-1}$ by 10.0% (65.1% to 58.6% of $\dot{V}O_2$max) and was associated with improved performance during this period (Conley, et al., 1984; Coyle, 2007; Daniels, 1985; Saunders, et al., 2004). Hence, it can be concluded that a high $\dot{V}O_2$max level is required for elite performance, but major improvements in RE, that may take years of training to achieve, may enable world-class performance. Additionally, the

blood lactate thresholds (i.e., the lactate threshold, the anaerobic threshold, the onset of blood lactate accumulation and the individual anaerobic threshold) (Coyle et al., 1991), the relative exercise intensity (i.e., the fraction of $\dot{V}O_2max$ used while running (%$\dot{V}O_2max$) (Kozlowski et al., 1985), and the histological factors (e.g., muscle capillarity and cross-sectional area determinations), are all key physiological principles for successful endurance running/exercise performance.

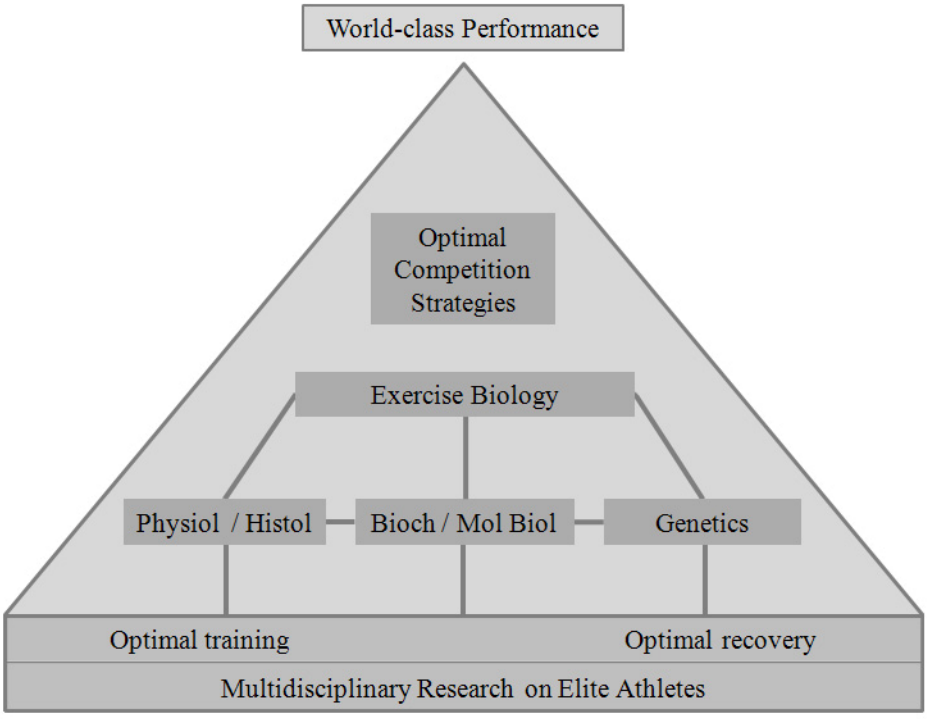


Figure 1.1 A multidisciplinary approach to research on elite athletes. Adapted from Myburgh (2003).

1.1.3 Multi-disciplinary approach and limitations

By way of explanation, it seems that several factors characterise the elite athlete and include: sustained effective training, a recovery program with effective nutritional support, a range of heritable and acquired characteristics, optimal competition strategies, while resistance to injury also appears to be important (Maughan, et al., 2004). The preceding factors have been discussed in scientific literature and are all currently being applied to questions relating to exercise capacity and the adaptation to exercise. However, the interactions between the aforementioned factors do not seem to be fully understood (Myburgh, 2003). Additionally, the lack of well accepted guidelines is a further limitation in understanding the factors affecting exercise performance (in order to achieve world-

class performance). The equivocal outcomes of the established recommendations on well-trained and elite athletes are due to two major limitations in the scientific research process. The first is the role of the commercial industry in scientific research process and the second one, concerns the design limitations of each study (e.g., the type and homogeneity of subjects, limited "real world" data). The following sections will address each of these issues in sequence.

1.2 The role of commercial industry in establishing guidelines

The formulation of guidelines entails collection of all relevant evidence. These pieces of evidence are then passed on to a group of experts who, through their knowledge and expertise, determine what needs to be done and the best way to do it (Moore, Derry, & Aldington, 2011). However, in many instances, established guidelines are not widely accepted for a variety of reasons (Bellis, 2011; Noakes, 2007a; Taylor & Giles, 2005). The aforementioned studies (Bellis, 2011; Noakes, 2007a; Taylor & Giles, 2005) expose two main concerns regarding established guidelines. In general, it appears that many guidelines lack an adequate, scientifically proven evidence base and have not been properly evaluated in appropriately controlled and randomized trials. This and the potential influence of commercial interests on scientific independence and objectivity seem to be limiting factors in establishing well accepted guidelines. For instance, Taylor and Giles (Taylor & Giles, 2005) exposed close ties between scientists and manufacturers and the findings are surprising (Figure 1.2). According to their study, more than one-third of authors who participate in the panels which publish the guidelines, declared financial links to affiliated pharmaceutical companies, with around 70% of panels being affected. In one case, every member of the panel had received payment by the company responsible for the drug that was ultimately recommended. Moreover, in the same survey (Taylor & Giles, 2005) more than 200 guidelines from around the world which were deposited with the US National Guideline Clearinghouse in 2004, were studied. Only 90 guidelines contained details concerning individual authors and their conflicts of interest, and of those, just 31 were free of commercial industry influence. These pieces of evidence above, clearly establish that guidelines should not be determined by individuals with financial conflicts of interest (Taylor & Giles, 2005). According to Noakes (2007a) the concerns discussed above are clearly applicable to the prevailing drinking guidelines (Sawka et al., 2007) drawn up by the American College of Sports Medicine (ACSM) as advocated by the Gatorade Sports Science Institute (GSSI). Gatorade and the GSSI are the only two

''platinum'' sponsors of the ACSM (Noakes, 2007a). The ACSM drinking guidelines promote the double recommendation that sports drinks containing salt (and glucose) are more beneficial during exercise than water and that drinking ''as much as tolerable'' is the preferred option during exercise. Both these conclusions favour the promotion of sports drinks, including Gatorade, over water for all who participate in physical activity (Noakes, 2007a). However the ACSM drinking guidelines contain no warnings of potential conflicts of interest, nor of receipt by either the ACSM or any of the authors of the guidelines, of financial or other ''rewards'' from the sports drinks industry.

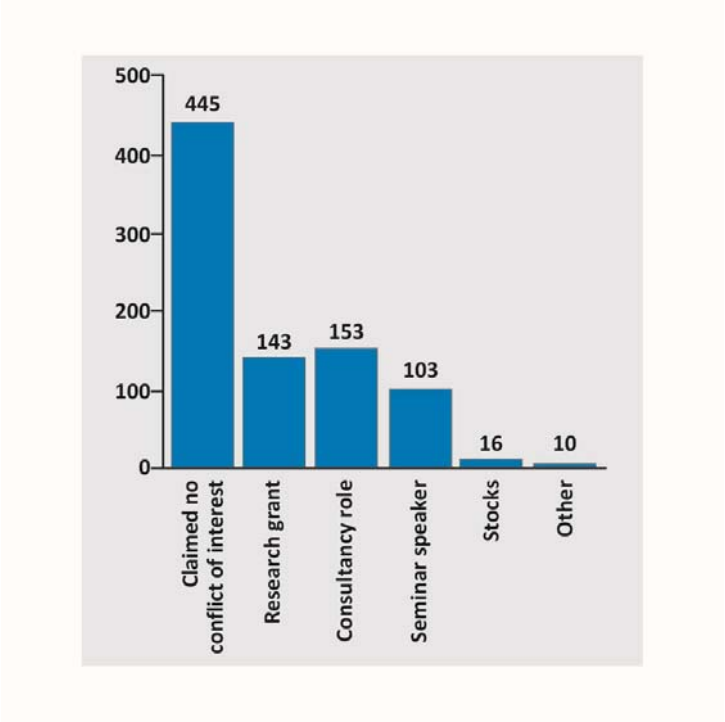


Figure 1.2 Conflicts of Interest. In 685 disclosures examined in survey of authors of prescription guidelines. Adapted from Taylor and Giles (2005).

Pharmaceutical research is not the only area influenced by industry. The other area of concern also influenced by the commercial industry, involves dietary supplements, which have become a popular item in the nutrition-related market. A dietary supplement is a product taken by mouth that contains a "dietary ingredient" intended to supplement the diet. The "dietary ingredients" in these products may include: vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites. Dietary supplements can also be extracts or concentrates, and can be found in a variety of forms such as tablets, capsules, soft-gels, gel-caps, liquids, or powders. They may also come in the form of a bar, but in such a case, the information on

their label must not represent the product as a conventional food or a sole item of a meal or diet. Whatever their form may be, the Dietary Supplement Health and Education Act (DSHEA) places dietary supplements into a special category under the general umbrella of "foods" not drugs, and requires that every supplement be labelled a dietary supplement (FDA, 2011).

For decades, the Food and Drug Administration (FDA), an agency of the United States Department of Health and Human Services, has protected the public from mislabelled and unsafe products. The FDA used to regulate the dietary supplements that contained only those essential nutrients listed in The Nutrition Labelling and Education Act of 1990. The new law (DSHEA, 1994), amended the Federal Food, Drug and Cosmetic Act, creating a new regulatory framework for the safety and labelling of dietary supplements. The DSHEA expanded the definition of dietary supplements so as to include other substances, not only essential nutrients. Dietary supplements are no longer considered food additives, which makes them exempt from pre-screening or from any safety and efficacy studies before they are released to the public. Unlike drug products that must be proven safe and effective for their intended use before being marketed, there are no provisions for FDA to "approve" dietary supplements for safety or effectiveness before they reach the consumer. Under DSHEA, it is the manufacturer who is responsible for determining that the dietary supplements manufactured or distributed are safe. When the DSHEA was approved, the FDA lost its regulatory power. According to industry data, the market for dietary supplements (vitamins, minerals, botanicals such as herbal products and other specialty products) in the USA was valued at $8.8 billion in 1994, then grew to $11.8 in 1997 (Ahrendt, 2001; Nesheim, 1999) to more than $15.7 in 2000 (Giunta, Basile, & Tibuzzi, 2010; Maughan, et al., 2004) and to $27 billion in 2009 (Figure 1.3) with more than 55600 products on the market (Abdel-Rahman et al., 2011). This growth has been attributed to a number of factors, including the DSHEA legislation of 1994, which made it easier to market dietary supplements with attractive properties, and the growth of the self-care movement (Giunta, Basile, & Tibuzzi, 2010). Indeed, the media may have contributed to the increase in the use of dietary supplements by spreading the myth of "the ideal body" as a way of convincing potential consumers to buy sports enhancement products (Alves & Lima, 2009; Maughan, et al., 2004). Notably, the global market for supplements in 2001 was estimated at $46 billion (Maughan, et al., 2004) while more than 50% of the general population and more than 76% of athletes in the US use nutritional supplements (Ahrendt, 2001). Therefore it is imperative to make a connection between the marketing of

supplements by the commercial industry and their consumption by athletes. As indicated previously, surveys show that the prevalence of supplement use is widespread among sportsmen and women, however only in few cases is the use of these products scientifically supported and some may even be harmful to the athlete (Maughan, et al., 2004). With broader use of a wide variety of supplements, greater concerns have arisen regarding the safety and quality of these products, since many may not have any scientific basis (Ahrendt, 2001). Some dietary supplement ingredients are unsafe for all consumers, while others should not be taken by certain segments of the population (e.g., children, elderly people) (Abdel-Rahman, et al., 2011). Additionally, poor manufacturing practices can result in products being contaminated with toxins, metal, or pesticides and in products containing too little or too much of the key ingredients (Abdel-Rahman, et al., 2011; Giunta, et al., 2010).

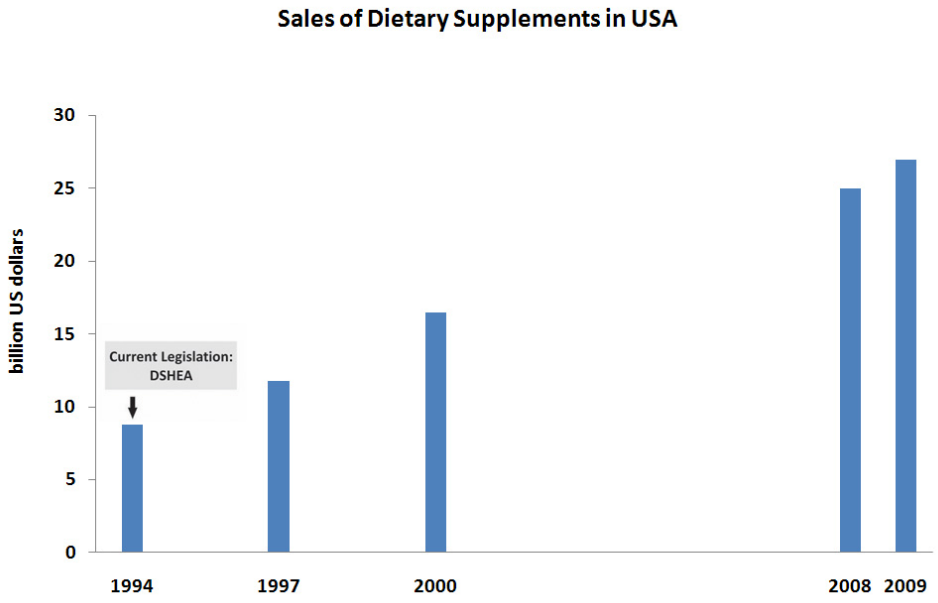


Figure 1.3 Sales of dietary supplements in USA

Many supplements are often referred to as ergogenic aids; specifically substances that enhance energy production and provide athletes with a competitive advantage (Ahrendt, 2001). As described previously, the marketing of ergogenic supplements is an international, billion dollar business that targets athletes' endeavour for excellence (ACSM, 2000). A published survey on random supplement costs is presented in Table 1.1. In general, an athlete may choose to consume a supplement for a number of reasons: in order to prevent or to resolve a specific nutritional deficiency (Alves & Lima, 2009)

especially when requirements for a nutrient are increased by the existing exercise programme, in order to provide a more convenient form of nutrients in situations where everyday foods are not practical, in order to provide a direct ergogenic (performance - enhancing) effect, or because of the belief that every top athlete is consuming the supplement, and consequently the athlete cannot afford to miss out (Burke, Castell, & Stear, 2009; Maughan, et al., 2004).

Table 1.1 Supplement costs based on a sampling of popular nutrition stores in January 2000 for one month's therapy at lowest usual dose.

Product	Cost ($)
Creatine powder	26
Creatine mixture	60
DHEA	14
Androstenediol	30
Androstenedione	30
HMB	100
Protein powder (50 g daily)	85
Antioxidant formulations	30
Energizing/diet capsules	35
Multivitamins	5 to 20

**Note.* DHEA, dehydroepiandrosteron. HMB, calcium beta-hydroxy beta-methylbutyrate.

*Adapted from Ahrendt 2001.

New products with ergogenic claims appear on the market almost daily. The need to produce novel supplements induces the industry to market "cocktails" made up of mixed ingredients in an attempt to maximize the potential effect of the product making it more attractive to athletes (Green, Catlin, & Starcevic, 2001). Although these new products claim to enhance sports performance, and are used by amateur and professional athletes (Ahrendt, 2001), many of them do not have the desired effect (Ahrendt, 2001). Therefore, before deciding to use a supplement, athletes should always consider the issues of efficacy, safety and legality/ethical issues associated with the product (Burke, et al., 2009). More specifically, athletes can evaluate these products by examining the following four factors; The method of action, the available research, the adverse effects, and the legality of the substance (Ahrendt, 2001). According to Ahrendt et al. (2001) and Kreider et al. (2004) the following questions can help athletes determine whether a product should be taken or not: 1. What is the physiological basis or theory for this product's action? 2. Are there any

scientific studies published in peer-reviewed journals that support or refute the claims made by the product? 3. Are there any side effects, especially any potentially serious adverse effects? 4. Is the product legal? Based on the above criteria, nutritional supplements can be placed into the following categories: apparently effective; possibly effective; too early to tell; and apparently ineffective (Kreider et al., 2004). Alternatively, the ACSM (ACSM, 2000) classifies dietary supplements into the following categories: those that perform as claimed; those that may perform as claimed but for which there is insufficient evidence of efficacy at this time; those that do not perform as claimed; and those which are dangerous, banned, or illegal, and consequently should not be used (ACSM, 2000). In any case, it is obvious that not all supplements have the effects they claim to have and in most cases there is opportunity for further scientific research in order to evaluate the potential effectiveness of the supplements (Table 1.2). Therefore, decisions about effectiveness must be extrapolated from the best available research.

Table 1.2 Categorization of the Ergogenic Value of Performance Enhancement, Muscle Building, and Weight Loss Supplements.

Category	Muscle Building Supplements	Weight Loss Supplements	Performance Enhancement
I. Apparently effective and generally safe	• Weight gain powders • Creatine • HMB (untrained individuals initiating training)	• Low-calorie foods, MRPs and RTDs that help individuals maintain a hypocaloric diet • Ephedra, caffeine, and salicin-containing thermogenic supplements taken at recommended doses in appropriate populations (now banned by FDA)	• Water and sports drinks • CHO • Creatine • Sodium phosphate • Sodium bicarbonate • Caffeine
II. Possibly effective	• Postexercise CHO and protein • BCAA • Essential amino acids (EAA) • Glutamine • Protein • HMB (trained subjects)	• High-fiber diets • Calcium • Phosphate • Green tea extract • Pyruvate/DHAP (at high doses)	• Postexercise CHO/PRO • Glutamine • EAA • BCAA • HMB (trained subjects) • Glycerol • Low doses of ephedrine/caffeine (now banned by FDA)
III. Too early to tell	• α-Ketoglutarate • α-Ketoisocaproate (KIC) • Ecdysterones • Growth hormone releasing peptides (GHRP) and secretogues • HMB (trained athletes) • Isoflavones • Sulfo-polysaccharides (myostatin inhibitors) • Zinc/magnesium aspartate (ZMA)	• Appetite suppressants and fat blockers (Gymnema sylvestre, chitosan) • Thermogenics (synephrine, thyroid stimulators, cayenne pepper, black pepper, ginger root) • Lipolytic nutrients (phosphatidyl choline, betaine, Coleus forskohlii, 7-keto DHEA) • Psychotropic Nutrients/Herbs	• Medium chain triglycerides • Ribose
IV. Apparently not effective and/or dangerous	• Boron • Chromium • Conjugated linoleic acids (CLA) • Gamma oryzanol (ferulic acid) • Prohormones • Tribulus terrestris • Vanadyl sulfate (vanadium) • Yohimbe (Yohimbine)	• Chromium (nondiabetics) • CLA • HCA • L-Carnitine • Pyruvate (at low doses) • Herbal diuretics • High doses of ephedrine/caffeine	• Inosine • High doses of ephedrine/caffeine

**Note.* Adapted from Kreider 2004.

While little scientific data is available to support the potential role of dietary supplements in training/performance enhancement a number of nutrients/dietary supplements have been confirmed to help in improving performance/recovery (Maughan, et al., 2004). For example, the effects of amino acid supplementation on physical performance have been widely investigated, with some studies showing positive effects on strength (Crowe, Weatherson, & Bowden, 2006; Schena, Guerrini, Tregnaghi, & Kayser, 1992) and endurance performance (Blomstrand, Hassmen, Ekblom, & Newsholme, 1991), while other studies report no ergogenic effect (Madsen, MacLean, Kiens, & Christensen, 1996; Pitkanen et al., 2003; van Hall, Raaymakers, Saris, & Wagenmakers, 1995). Despite conflicting results, amino acid supplementation amongst athletes is widespread (Ahrendt, 2001; Maughan, et al., 2004), with most of them believing that exercise performance will be enhanced. Given the lack of well-controlled studies on nutritional supplements conducted on well-trained subjects, opportunity for further research is available. A good example is GAKIC; a specific combination of amino acids, namely glycine, arginine and α-ketoisocaproic acid (a breakdown product of leucine). GAKIC is marketed as "the world's first and only muscle fatigue toxic neutralizer" (GAKIC, 2010). There are only two available scientific studies that have examined the effects of GAKIC supplementation on human performance during high intensity and short duration exercise (Buford & Koch, 2004; Stevens, Godfrey, Kaminski, & Braith, 2000). Both studies employed untrained individuals as subjects. Although these are the only two studies that had examined the effects of GAKIC on exercise performance, both investigations showed ergogenic effects but not any clear mechanism(s), a fact that suggests that neither study is definitive. Given the scientific limitations in the design of both existing studies (Buford & Koch, 2004; Stevens, et al., 2000) this commercial product could be categorized in the "too early to tell" class of supplements (Table 1.2) thus allowing for this potential sport enhancing aid to warrant further investigation.

1.3 Properly evaluated and controlled studies in guidelines establishment

1.3.1 Limited scientific evidence based data conducted on food and fluid intake on a homogeneous group of athletes

The potential influence of commercial interests on scientific subjectivity is described in section 1.2. The other consideration and possible source of conflict is the means by which proper guidelines are established do not serve conflicting objectives. On examination there

are approximately 33600 scientific publications in Pub Med that include the term 'guidelines' in the title, and almost 1000 with the addition of 'evidence-based'. However, when a guideline claims to be evidence-based it does not necessarily mean that it offers the correct advice (Moore, et al., 2011). To expand the Pub Med search, keywords such as "world-class athletes", "world-class performance", "elite athletes' nutrition", "elite athletes' hydration", and "highly trained athletes" were used. The literature found in the search included consensuses, editorials, original articles, and review articles written in English. The search revealed a word combination of no more than 300 studies (i.e., highly trained athletes-346) while, a vast number of the titles were not directly related to the objectives of the current thesis. For example, the majority of peer-reviewed articles cited in the guidelines did not investigate a homogeneous group of athletes (e.g., either well-trained, or elite, or world-class), rendering their conclusions somewhat generalised for the current conundrum. Therefore, studies examining "real world" data, which indicate what the best athletes eat and drink, are imperative in order to assess and possibly improve the applicability of the current recommendations for dietary intake.

The American Dietetic Association, Dieticians of Canada, and the ACSM state that physical activity, athletic performance, and recovery from exercise are enhanced by optimal nutrition (Rodriguez, Di Marco, & Langley, 2009). These organizations recommend appropriate selection of food and fluids, timing of intake, and supplement choices for optimal health and exercise performance (Rodriguez, et al., 2009). Furthermore, to maximize training effects and maintain body mass (BM) and health, athletes need to consume adequate energy during periods of high-intensity and/or long-duration training. Meeting energy needs should be a nutritional priority for optimum athletic performance (Rodriguez, et al., 2009). Restricting energy intake (EI) or adopting severe weight loss practices, results in the elimination of one or more food groups from the diet or in the risk of micronutrient deficiencies due to consumption of high or low carbohydrate (CHO) diets of low micronutrient density. Another consensus statement on Nutrition for athletics published by the International Association of Athletic Federations (IAAF) in 2007 states: "Well chosen foods will help athletes train hard, reduce risk of illness and injury, and achieve performance goals, regardless of the diversity of events, environments, nationality and level of competitors." (IAAF, 2007). Such consensus has emerged by taking into account over a century of diet/metabolic research where nutrition has been clearly documented to have beneficial effects on exercise performance. It is evident that the foods chosen can make the difference between success and failure

(Maughan, et al., 2004). This of course does not imply that a non-talented or non-motivated athlete will become a champion, but signifies that a talented athlete may be prevented from making it to the top by following an inadequate diet (Maughan, et al., 2004). The athlete who wants to optimize exercise performance needs to follow correct nutrition and hydration practices, use supplements and ergogenic aids with care, and consume a variety of foods in adequate amounts (ACSM, 2000; Below, Mora-Rodriguez, Gonzalez-Alonso, & Coyle, 1995; Brooks & Mercier, 1994; Calders, Matthys, Derave, & Pannier, 1999; Coggan & Coyle, 1991; Jeukendrup, Brouns, Wagenmakers, & Saris, 1997). Thus, making considerate choices about the type, timing, and quantity of food to be consumed as well as considering the competition schedule, can all play a role in optimizing exercise performance.

There is no doubt that what an athlete eats and drinks can affect health, body weight and composition, substrate availability during exercise, recovery time after exercise, and ultimately exercise performance (ACSM, 2000). Nevertheless, the "real world" evidence data regarding the nutritional needs of world-class athletes for endurance running are limited. Therefore, the establishment of specific nutritional recommendations requires further investigation. The nutritional guidelines should be practical and direct in order to be followed correctly by athletes. Hence, more research involving world-class athletes is required in order to improve existing guidelines. Certainly, elite East African runners are an appropriate target group for investigating this area, as they are a major dominant force in endurance running (IAAF, 2007). Therefore, the East African running phenomenon is unequivocal in the world's competitive athletics. It should be noted that there have been periods in history when other countries or groups of athletes have also had great success in distance running. However, these were the accomplishments of individual athletes and do not constitute a general phenomenon as do East African runners. Nevertheless, there are only five studies that have assessed the dietary practices of elite East African runners and all studies have involved Kenyan athletes (Christensen, Van Hall, & Hambraeus, 2002; Fudge et al., 2008; Fudge et al., 2006b; Mukeshi & Thairu, 1993; Onywera, Kiplamai, Boit, & Pitsiladis, 2004). Since historically there has been very little cultural or biological exchange between Kenyans and Ethiopians (Scott et al., 2009), it is hypothesized that there may be distinct dietary habits adopted in the two countries. Therefore, there is a need for novel data that can be obtained from some of the best athletes in Ethiopia in order to assess and possibly improve the applicability of the current recommendations to elite athletes, in particular endurance runners.

1.3.2 Concerning the transfer of data from lab to field and the establishment of reasonable debates

A rationale for debate is data transference from laboratory-based investigations to the field setting (Saunders, Dugas, Tucker, Lambert, & Noakes, 2005). The tremendous debate regarding the most effective fluid intake strategy for endurance athletes is a good example (Convertino et al., 1996; Noakes, 2001). Based on laboratory experiments (Costill, Kammer, & Fisher, 1970; Gisolfi & Copping, 1974; Montain & Coyle, 1992) the ACSM (Convertino, et al., 1996) adopted the previous fluid replacement guidelines that advocate ingesting large amounts of fluid in order to prevent any weight loss during exercise. In contrast to the aforementioned conclusion by the ACSM (Convertino, et al., 1996), Saunders et al. (2005) proposed that the three laboratory studies (Costill, et al., 1970; Gisolfi & Copping, 1974; Montain & Coyle, 1992) which report a relationship between increased levels of heat storage and dehydration, are performed in conditions of low wind-speed which negatively affect the rate of heat loss in the previously mentioned studies. More specifically, convective and evaporative cooling was likely to be less than that encountered in outdoor competition. Therefore, reduction in the heat dissipation potential is associated with convective and evaporative heat transfer coefficients (Cheuvront, Carter, Montain, Stephenson, & Sawka, 2004). In general, Saunders et al. (2005) support that laboratory studies in cycling and running, which employ wind speeds that are substantially lower than an athlete's estimated cycling or running speed should not be used as a basis for establishing fluid replacement guidelines, since they underestimate the body's ability to adapt to mild dehydration and overestimate the beneficial effects of high rates of fluid ingestion (Saunders, et al., 2005). For instance, Adams et al. (1992) found that the subjects studied had higher body temperatures (rectal and oesophageal) in wind-still conditions of 0.75 $km \cdot h^{-1}$ compared to a facing wind velocity of 11.25 $km \cdot h^{-1}$; consequently the cooling rate may be different outdoor than in a laboratory. In general, laboratory studies examining the biophysical consequences of hypo-hydration when working in a hot environment provide less airflow than what is naturally generated when running or cycling over ground (Saunders, et al., 2005). Empirical observations support the notion of the failure of laboratory experiments to simulate "outdoor" conditions (for a review see reference (Cheuvront & Haymes, 2001a). Consequently, data for establishing universal guidelines must be extracted from studies that are properly evaluated in appropriately controlled, randomized trials conducted under environmental and other conditions that match those found in ''outdoor'' exercise (Noakes, 2007a).

One of the most scientifically equivocal research areas is the "science of hydration". A further example is the lively debate concerning dehydration (i.e., the loss of body water or the process of reducing body water) and its effects on exercise performance (Sawka & Noakes, 2007). Nevertheless, the recommended guidelines for specific fluid intake of the ASCM in 1987 are considered to be the prevailing ones (Binkley, Beckett, Casa, Kleiner, & Plummer, 2002; Convertino, et al., 1996; Montain, Latzka, & Sawka, 1999). These guidelines are regularly revised and adjusted accordingly. Analytically, fluid intake recommendations for long-distance events such as the marathon have changed substantially over the last half century. During the first half of the twentieth century until the early 1970s, runners were typically advised to avoid ingesting fluid during competitive marathon running (Noakes, 2003b). However, since the publication of the 1987 ACSM position stand on fluid intake, recommendations have typically advocated that all sweat loss during exercise should be replaced by drinking the maximum amounts that can be tolerated (Binkley, et al., 2002; Convertino, et al., 1996; Montain, et al., 1999). More recently, the ACSM has replaced the 1996 Position Stand (Convertino, et al., 1996) with an updated version (Sawka, et al., 2007) that promotes drinking *ad libitum* (i.e., at one's pleasure) from 0.4 to 0.8 $L \cdot h^{-1}$ of fluid during exercise. The most recent guideline advises the prevention of a weight loss of more than 2-3% BM due to dehydration, and recommends this specific range of fluid ingestion (i.e., 0.4 to 0.8 $L \cdot h^{-1}$) depending on BM and running speed (Sawka, et al., 2007). The efficacy of proposing a specific fluid intake range during exercise has been investigated in a study that modelled parameters that influence sweating rate (Montain, Cheuvront, & Sawka, 2006). Montain et al. (2006) estimated that this rate (i.e., 0.4 – 0.8 $L \cdot h^{-1}$) of fluid intake was sufficient to maintain BM loss within 3% and to prevent BM gain in 50 – 90 kg subjects running a marathon at 8.5 – 15 $km \cdot h^{-1}$ in cool and warm ambient conditions (i.e., 18 ºC and 28 ºC, respectively). However, in their calculations, these authors did not consider the fluid requirements of elite marathon runners (Montain, et al., 2006), making the existing debate reasonable (Sawka & Noakes, 2007). For example, maximum running speed calculated was 15 $km \cdot h^{-1}$, whereas to win a major city marathon a male athlete must run faster than 19 $km \cdot h^{-1}$. Furthermore, the environmental temperatures (i.e., 18 ºC and 28 ºC) on the basis of which sweat rates and resultant BM loss were estimated do not adequately reflect the lower environmental temperatures typically experienced in most major city marathons (Cheuvront & Haymes, 2001b) or indeed the ambient conditions encountered when the fastest marathons are run (Fudge, Pitsiladis, Kingsmore, Noakes & Kayser, 2006a). This observation is not unexpected given the laboratory results of (Galloway & Maughan, 1997)

that found the longest exercise duration at 10.5 ºC (93.5 ± 6.2 min) vs. 3.6 °C (81.4 ± 9.6 min) vs. 20.6 °C (81.2 ± 5.7 min) vs. 30.5 ºC (51.6 ± 3.7 min). The progress of the guidelines on hydration over the years is presented in Figure 1.4.

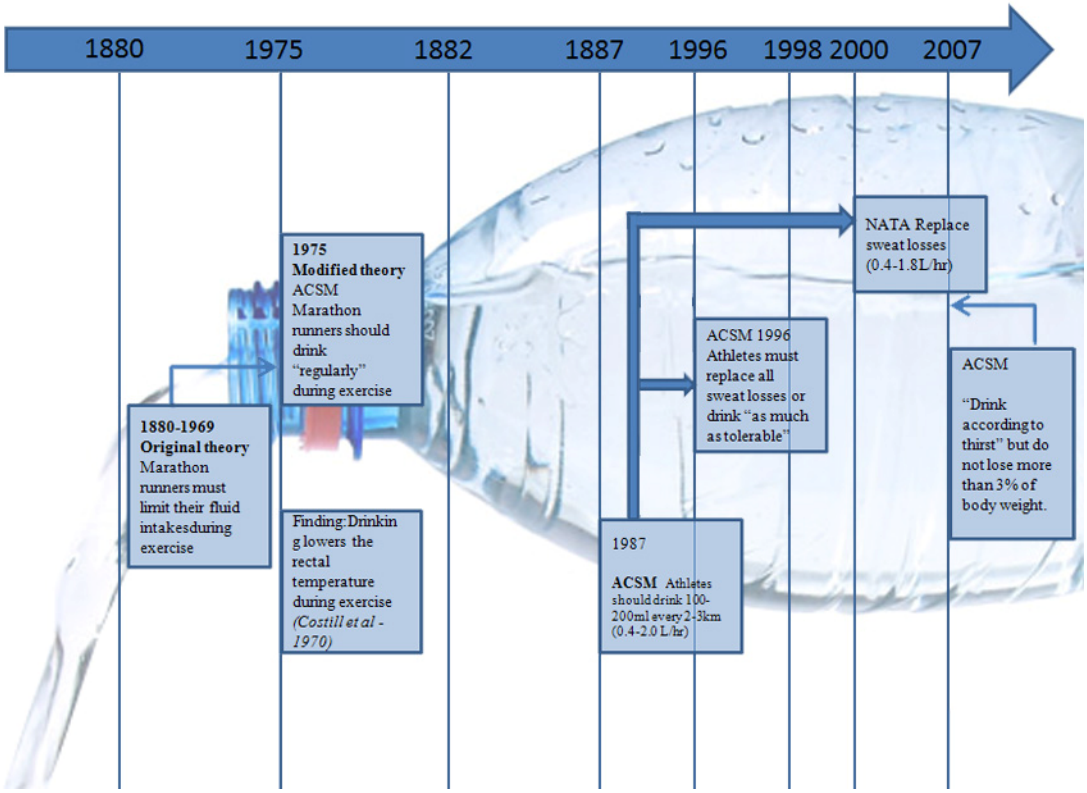


Figure 1.4 The progress of the guidelines of hydration over the years.

Athletes are advised to drink enough so as to ensure that they do not lose more than 2% of BM, based on the premise that BM loss greater than 2-3% impairs exercise performance (Sawka & Noakes, 2007). Indeed, there are many studies supporting that correct fluid replacement practices are especially crucial in endurance events and water seems to be the most important nutritional ergogenic aid for athletes as a body water deficit in excess of 2-3% BM marks the level of dehydration that can adversely affect performance (Cheuvront, Carter, & Sawka, 2003; Sawka, 1992). Thus, adequate fluid intake before, during, and after exercise is important for health and optimal performance (Rodriguez, et al., 2009). Additionally, many other studies support that mental performance (Hancock & Vasmatzidis, 2003) as well as the subjective perception of the exercise difficulty (Montain & Coyle, 1992) are affected negatively by a body water deficit of more than 2-3% of BM. On the other hand, there is data to support that there is no real evidence that drinking more than *ad libitum* improves exercise performance regardless of the extent of the BM loss. A meta-analysis of all laboratory studies of fluid ingestion during self-paced exercise

concludes that "exercise-induced dehydration does not alter cycling performance under "real world" or "outdoors" exercise conditions (Goulet, 2011). Additionally, as far as hyperthermia is concerned; it seems unlikely to occur in prolonged competitive events held in mild environmental conditions, and that exercise intensity rather than the level of dehydration is probably the most important factor determining post-exercise rectal temperature (Noakes et al., 1988). The lack of evidence on what the best endurance runners drink during running competition seems to be a major limitation in establishing the guidelines since currently there exist limited data on the drinking behaviours of the fastest marathon runners during competition (Cheuvront & Haymes, 2001b; Van Rooyen, Hew-Butler, & Noakes, 2010; Zouhal, et al., 2010). If these data concerning drinking behaviours of the best endurance runners during running competition existed, this would allow for the adequacy of current fluid intake recommendations (Sawka & Noakes, 2007) to be assessed.

1.4 Hydration in hot and humid environment: An example of bringing together "real world" and guidelines

It has been shown that athletes who lose the most BM during marathon and ultra-marathon races and ironman triathlons are usually the most successful (Cheuvront & Haymes, 2001b; Muir, Percy-Robb, Davidson, Walsh, & Passmore, 1970; Pugh, Corbett, & Johnson, 1967; Sharwood, Collins, Goedecke, Wilson, & Noakes, 2004; Wharam et al., 2006; Wyndham & Strydom, 1969; Zouhal, et al., 2010; Zouhal et al., 2009). However, the prevailing guidelines propose a water deficit of no more than 3% of BM in order to preserve cardiovascular and thermoregulatory function during exercise (Sawka, et al., 2007). This limit is often exceeded since the proposed recommendations for fluid consumption during a race (Sawka & Noakes, 2007) are not always practical for various reasons (e.g., difficulties of providing drinks during a race, athletes' difficulty to drink while running), preventing the athlete from consuming the recommended amounts of fluids. Additionally, sweat loss has been shown to be higher than fluid intake leading to dehydration. Although, lively debate exists in the literature on whether dehydration negatively affects performance or not (Sawka & Noakes, 2007), the rationale for fluid ingestion during exercise seems the most appropriate practice so as to achieve the minimum water deficit (i.e., < 3%). This level of dehydration seems to compromise cardiac output and the capacity of the cardiovascular system to transport metabolic heat to the body surface (Casa et al., 2000; Montain & Coyle, 1992) and to increase the risk of heat exhaustion during and immediately after activity (Montain & Coyle, 1993).

Therefore, maintaining hydration at less than a 3% of BM during exercise helps preserve heart rate (HR), stroke volume, cardiac output, skin blood flow, and normal core temperature (T_{core}) (Armstrong et al., 1997). Therefore given the fact that athletes do not consume enough liquids during a race, a better hydrating strategy prior to the race would potentially be more advantageous. The implementation of a well regulated water loading strategy with the use of hydrating agents, prior to exercise, in order to expand the water compartments and consequently maintain cardiovascular and thermoregulatory responses is imperative. In this way, the recommended guidelines could find practical application since one may argue that even the fastest marathon runners can improve their exercise performance achievement by following the guidelines.

Hydrating agents such as glycerol (Gly; 1,2,3-propanetriol) and creatine (Cr) have shown to have significant hydrating effects, although the exact mechanisms remain to be elucidated. Gly is a 3-carbon sugar alcohol that provides the backbone of triglycerides and is naturally found in foods as a component of dietary fats. When consumed simultaneously with a substantial volume of fluid, there is a temporary retention of this fluid and expansion of body fluid compartments. Rosendal et al. (2010) have recently reviewed the literature regarding Gly use. Effective protocols for gly hyper-hydration are 1–1.5 $g \cdot kg^{-1}$ BM with an intake of 1.4 - 2.0 L of fluid 2.5 - 4 h before exercise (Anderson, Cotter, Garnham, Casley, & Febbraio, 2001; Lyons, Riedesel, Meuli, & Chick, 1990). Such a protocol typically achieves a fluid expansion or retention of approximately 400–800 mL (Kern, Podewils, Vukovich, & M.J., 2001; Magal et al., 2003; Riedesel, Allen, Peake, & Al-Qattan, 1987; Volek et al., 2001) above a matched fluid dose via a reduction in urinary volume (Poortmans, Rawson, Burke, Stear, & Castell, 2011). This results in reduced thermal and cardiovascular strain during exercise in the heat (Easton, Turner, & Pitsiladis, 2007). It should be noted that Gly properties are also related to plasma expansion (Murray, Eddy, Paul, Seifert, & Halaby, 1991; van Rosendal, Osborne, Fassett, & Coombes, 2010). For this reason, the World Anti-Doping Agency (WADA) has added Gly to the group of 'masking agents' in the 2010 Prohibited List (WADA, 2010). However, the scientific basis of the inclusion of Gly as a 'masking agent' remains to be conclusively determined as several published studies have indicated that no changes in haemoglobin (Hb), haematocrite (Hct) or serum electrolyte concentrations are elicited by Gly ingestion (Lyons, et al., 1990).

Unlike the overall-body-hydrating effects of Gly, ingestion of 20 $g \cdot day^{-1}$ of Cr dissolved in 500 mL of water for 7 days has been shown to be effective in retaining fluid predominantly in the intracellular fluid compartments (Easton, et al., 2007; Kern, et al., 2001; Kilduff et al., 2004) resulting in an increased specific heat capacity of the body (Kern, et al., 2001; Kilduff et al., 2004) and consequently attenuating the rise in HR and T_{core} during exercise in the heat. Easton et al. (2007) were the first to add Gly to a Cr containing solution and demonstrated a combination of the two hyper-hydrating agents has an additive effect, as the addition of Gly to Cr significantly increased total body water (TBW) more than Cr alone. This novel water-loading strategy that combines Cr and Gly resulted in a mean TBW increase of approximately 0.9 ± 0.2 L, a significantly larger volume than either Cr or Gly alone. In addition, the retained water was dispersed equally between the intracellular water (ICW) and extracellular water (ECW) compartments resulting in the reduction of cardiovascular and thermoregulatory responses during exercise. Despite the physiological benefits accompanied by the TBW increase, this rise in the body water compartments leads to a subsequent rise in BM. This, in turn, may have a negative impact on RE and thus on performance (Easton, et al., 2007). As described in section 1.1, exercise performance is influenced by several factors such as RE which may be affected by BM which in turn is closely related to hydration status. The more water in the body the higher the BM is. Presumably during running, the greater the BM is, the greater the oxygen demand of the skeletal muscle is since more weight has to be moved when running. If this sequence holds true, hydration status may have an impact on performance. Therefore, a need to investigate the effects of hyper-hydration induced by a combined Cr and Gly supplementation on RE measures should be investigated further.

1.5 Aims and Objectives

Given the potential role of the commercial industry and the limited properly evaluated and controlled data derived in "real world" conditions concerning the dietary (foods and liquids) recommendations and nutritional supplementation (as factors for optimal training and recovery) for elite athletes, the main objectives of the following research were:

1. To compare data extracted from a well-controlled study to previous investigations that did not control for primary confounding factors that could have adversely affected the results. This will be investigated by examining the effects of a nutritional supplementation (GAKIC) on fatigue during high-intensity, repeated cycle sprints in trained cyclists.

2. To examine the application of established guidelines for food and fluid intake on an elite group of endurance runners. This will be achieved by assessing the food and macronutrient intake of elite Ethiopian distance runners during a period of high-intensity exercise training at altitude and prior to major competition. The data will also be compared to previous studies conducted on East African runners.

3. To describe the drinking behaviours of elite male marathon runners during major city marathons. The results from the current study will allow direct comparison to established guidelines on optimal hydration during long distance running.

4. To investigate the effects of a hyper-hydration strategy that combines Cr and Gly supplementation, on thermoregulatory responses in hot and cool conditions. The data will allow examination of a possible method that will bring together the established guidelines and the data extracted from "real world" studies.

2. Failure of Glycine-arginine-alpha-ketoisocaproic to improve high intensity exercise performance in trained cyclists

2.1 Introduction

The effects of amino acid supplementation on physical performance have been widely investigated with some studies showing positive effects on strength (Crowe, Weatherson, & Bowden, 2006; Schena, Guerrini, Tregnaghi, & Kayser, 1992) and endurance performance (Blomstrand, et al., 1991), while other studies report no ergogenic effect (Madsen, MacLean, Kiens, & Christensen, 1996; Pitkanen et al., 2003; Van Hall, Raaymakers, Saris, & Wagenmakers, 1995). Despite conflicting results, amino acid supplementation amongst athletes is widespread (Ahrendt, 2001; Maughan, et al., 2004) in the belief that exercise performance will be enhanced. Two studies have examined the effects of GAKIC on human performance during high intensity short duration exercise (Buford & Koch, 2004; Stevens, Godfrey, Kaminski, & Braith, 2000). In the first study, Stevens, et al. (2000) measured peak torque and work values in the quadriceps femoris of 13 subjects using a computer-controlled isokinetic dynamometer, over a 23-days interval. These authors (Stevens, et al., 2000) found that GAKIC supplementation increased muscle torque and work sustained during intense acute anaerobic dynamic exercise and increased overall muscle performance by delaying muscle fatigue during the early phases of anaerobic dynamic exercise. A subsequent study by Buford & Koch (2004), appeared to support the previous findings (Stevens, et al., 2000). In the latter study (Buford & Koch, 2004) 10 men completed a randomized, double-blinded, placebo-controlled exercise protocol of two sessions separated by 7 days. Subjects consumed either 11.2 g GAKIC or Pl during a 45-min period prior the exercise trials. Mean power, peak power, and fatigue values were assessed from 5 supra-maximal, 10-s cycle ergometer sprints, separated by 1-min rest intervals. It was found, that GAKIC supplementation attenuated the decline in mean power output during repeated cycling sprints. Both studies employed untrained individuals as subjects. Although these are the only two studies to have examined the effects of GAKIC on exercise performance, both report ergogenic effects yet neither seem to provide a clear mechanism(s) by which the components of GAKIC act (individually or synergistically). The amino acids making up GAKIC appear to be active in the metabolic pathways associated with the biosynthesis of Cr, protein, and nitric oxide (Campbell, La

Bounty, & Roberts, 2004; Minuskin, Lavine, Ulman, & Fisher, 1981; Nair, Schwartz, & Welle, 1992). The effects of GAKIC may be to stabilize muscle pH and/or attenuate the rise in ammonia concentration (Greenstein, Birnbaum, Gullino, Otey, & Winitz, 1956) released from the purine nucleotide cycle (Meyer & Terjung, 1979; Terjung, Dudley, & Meyer, 1985) possibly through alterations in the enzymatic pathways of nitrogen metabolism (Jeevanandam, Ali, Holaday, Weis, & Petersen, 1993; Sitren & Fisher, 1977). As such, GAKIC could enhance high intensity exercise performance as ammonia accumulation has been implicated in fatigue (Wilkinson, Smeeton, & Watt, 2010). GAKIC is marketed as "Muscle Fatigue Toxin Neutralizer" (GAKIC, 2010) and appears to render an ergogenic effect within minutes of consumption. This presents an obvious advantage for GAKIC supplementation (Buford & Koch, 2004).

Although previous research indicates GAKIC supplementation improves high intensity exercise performance (Buford & Koch, 2004; Stevens, et al., 2000), the paucity of data warrants further investigation. Furthermore, both previous studies (Buford & Koch, 2004; Stevens, et al., 2000) used untrained subjects and failed to incorporate more than one baseline trial leaving the reliability of the data open to question. The subjects in the study by Buford & Koch (2004) completed a modified Wingate test consisting of 5 sprints of 10 s. Although there were no statistical differences in peak power output following GAKIC supplementation, an increased power output was reported in the last of the 5 sprints. Conceivably, had the experiment been continued to include further sprints, an attenuation in the decline in peak power output may have been observed following GAKIC supplementation. Therefore, the aim of this Chapter was to investigate the effects of GAKIC on fatigue during a series of 10 sprints in trained cyclists using near identical experimental design as the study by Buford & Koch (2004). It was hypothesized that GAKIC supplementation would enhance peak power output and attenuate the decline in power output during repeated sprints in trained cyclists concurrent with previous research.

2.2 Methods

2.2.1 Subjects

Ten trained male cyclists (age 33 ± 6 years, weight 72.4 ± 8.9 kg, height 176 ± 1 cm) gave their written informed consent to take part in the present study which was approved by the local Ethics Committee and was performed according to the code of ethics of the World

Medical Association (Declaration of Helsinki). The sample size of the present study was in accordance with the subject numbers used in the two previous studies involving GAKIC (Buford & Koch (2004); Stevens, et al., 2001) as well as the majority of previous relevant studies (see Hopkins, Schabort, & Hawley, 2001 for review). Subjects were in good health at the time of testing, trained almost daily and participated regularly in local and national races.

The overall design was a randomized, cross-over scheme conducted in a double-blind fashion, with trained cyclists completing two performance trials consisting of 10 repeated supra-maximal sprints of 10 s. All tests were conducted on an electrically braked cycle ergometer (Lode Excalibur, Lode BV, Groningen, The Netherlands) at standard room temperature (19 – 21 ^{o}C) with one week separating each test. Prior to these performance trials, familiarization trials were completed until the variability of peak power output was within 5% difference between the two consecutive trials. No subject had to complete a third familiarization trial to achieve less than 5% variability in line with our previous experience of trained cyclists. Subsequently, all subjects completed two performance trials prior to which they ingested either GAKIC or placebo (Pl) in a double-blinded crossover fashion and in accordance with previous studies (Buford & Koch, 2004; Stevens, et al., 2000).

2.2.2 Experimental Procedures

Subjects reported to the laboratory on the day of testing following a 3 h fast and having refrained from alcohol and strenuous exercise the day before. Upon arrival at the laboratory, BM (Avery Weight-Tronix 3302, ABN, Birmingham, U.K.) and height (Invicta Plastics Ltd., Leicester, U.K.) were measured. Additionally, a HR monitor (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) was attached prior to each test. Subjects were also required to complete a 24-h dietary record and reproduce this diet the day prior to each trial in an attempt to standardize the diet in terms of total energy intake (TEI), macronutrient intake and quantity of specific amino acids (glycine, arginine and leucine).

Each subject ingested either 11.2 g of commercially available GAKIC (2.0 g glycine, 6.0 g L-arginine monohydrochloride, 3.2 g α-ketoisocaproic acid calcium salt; Iovate Health Sciences Research Inc., Mississauga, ON, Canada) or Pl (9.46 g sucrose, 3.2 g calcium carbonate) prior to the experimental performance trials. No attempt was made to verify the

presence of the active ingredients. Supplements were dissolved in 450 mL of sugar free fruit juice and subjects were required to wear a nose clip to prevent differentiating between beverages as GAKIC has a distinguishable and fairly unpleasant odor. It was confirmed via a simple discussion at the end of each experiment that subjects were not able to identify any differences between the treatments. The supplement was divided into 3 equal aliquots of 150 mL and ingested 45, 30 and 10 min prior to exercise. Timing of ingestion and supplementation dosage was in line with those used in the two previous studies (Buford & Koch, 2004; Stevens, et al., 2000). After ingestion of the third and final beverage, subjects commenced a warm-up consisting of 5 min of cycling at 100-150 W, followed by a familiarization sprint of 5 s. The warm-up also consisted of stretching exercises depending on each individual. Subsequently, subjects performed a series of ten, maximal effort 10 s sprints, each separated by a 50 s rest interval. Each sprint was a modified 10 s Wingate test performed using a resistance of 0.8 $N \cdot kg^{-1}$. Power output was analysed using standard computer software (Version 1, Lode BV, Groningen, The Netherlands). This analysis enabled calculation of both 10 s mean power (the average power output during each 10 s sprint) and peak power output (the highest power during each sprint). Furthermore, the fatigue index was determined as follows: fatigue index % = [(peak power – minimum power)/peak power] x 100 where the minimum power is the lowest power output after the subject achieved peak power output. After the 10 sprints, subjects were instructed to continue pedalling for at least 6-7 min to allow recovery to occur. HR was recorded (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) and ratings of perceived exertion (RPE) determined using the Borg category scale (Borg, 1982) at the end of each sprint. Illustrations of the lab work are presented in Figure 2.1.

Figure 2.1 Illustration of the lab work data collection.

2.2.4 Data analysis

All data are expressed as the mean ± SD. Following a test for the normality of distribution all repeated measures data (i.e., peak power, mean power, minimum power, fatigue index, HR, and RPE were compared between the two treatments using a two-way repeated measures analysis of variance (ANOVA). Other comparisons (i.e., total energy and macronutrient) were compared using students paired t-test. Statistical significance was set at $P < 0.05$. All statistical analysis was completed using the software package SPSS, version 11.0 (SPSS, Inc., Chicago, IL, USA).

2.3 Results

There were no significant differences in either macronutrient or micronutrient intake prior to either GAKIC or Pl trials. Detailed diet analysis is presented in Table 2.1. No significant differences were observed between GAKIC and Pl treatments in any of the measured performance variables. Peak power (Figure 2.2) declined from the 1st sprint (Pl:

1332 ± 307 W, GAKIC: 1367 ± 342 W) to the 10th sprint (Pl: 1091 ± 229 W, GAKIC: 1061 ± 272 W) and did not differ significantly between conditions (P = 0.88). Mean power (Figure 2.3) declined from the 1st sprint (Pl: 892 ± 151 W, GAKIC: 892 ± 153 W) to the 10th sprint (Pl: 766 ± 120 W, GAKIC: 752 ± 138 W) and did not differ between conditions (P=0.96); the average coefficient of variation (C.V.) for the 10 sprints taking into account both trials was 2.1% and 2.2% for peak and mean power output, respectively (calculated as indicated in Hopkins, et al., 2001). In general, the fatigue index (Figure 2.4) remained at ~38% throughout the series of sprints and did not differ between conditions (P = 0.99).

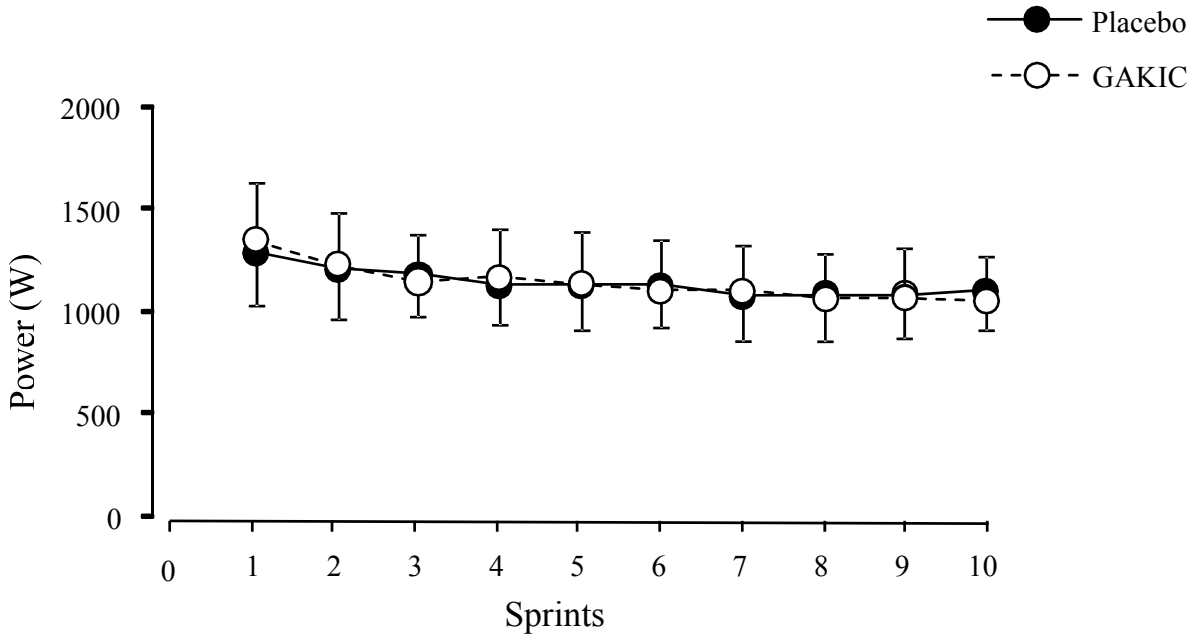


Figure 2.2 Peak power outputs over ten repeated cycling sprints with GAKIC and Pl treatments (*N* = 10, mean ± SD).

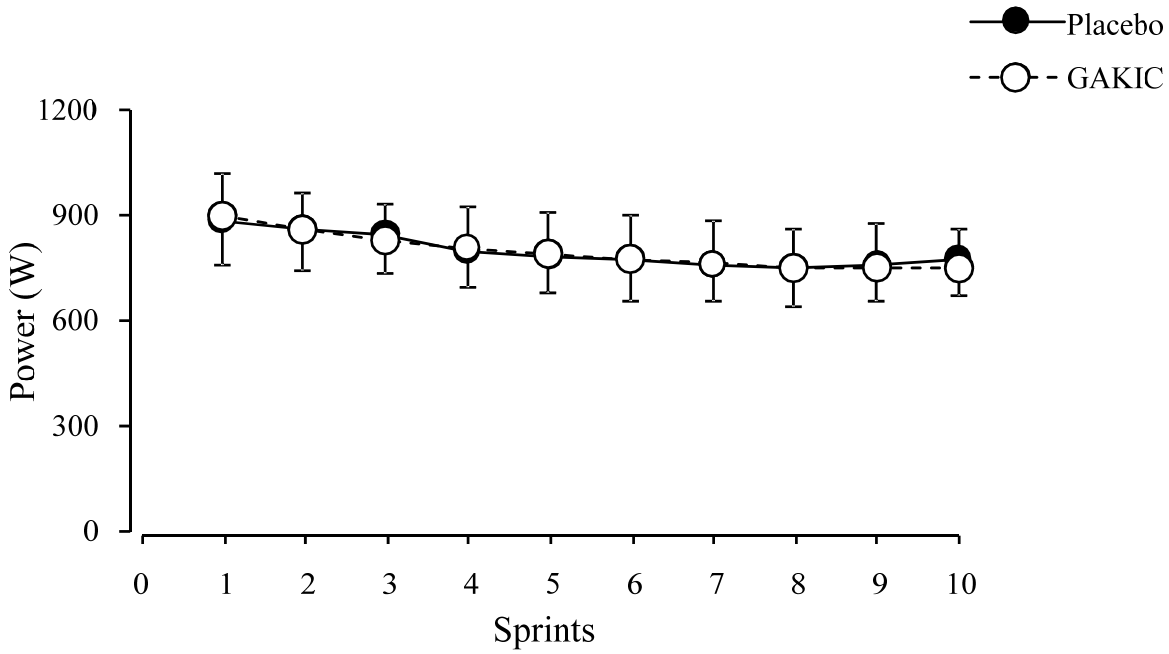


Figure 2.3 Mean power outputs over ten repeated cycling sprints with GAKIC and Pl treatments (*N* = 10, mean ± SD).

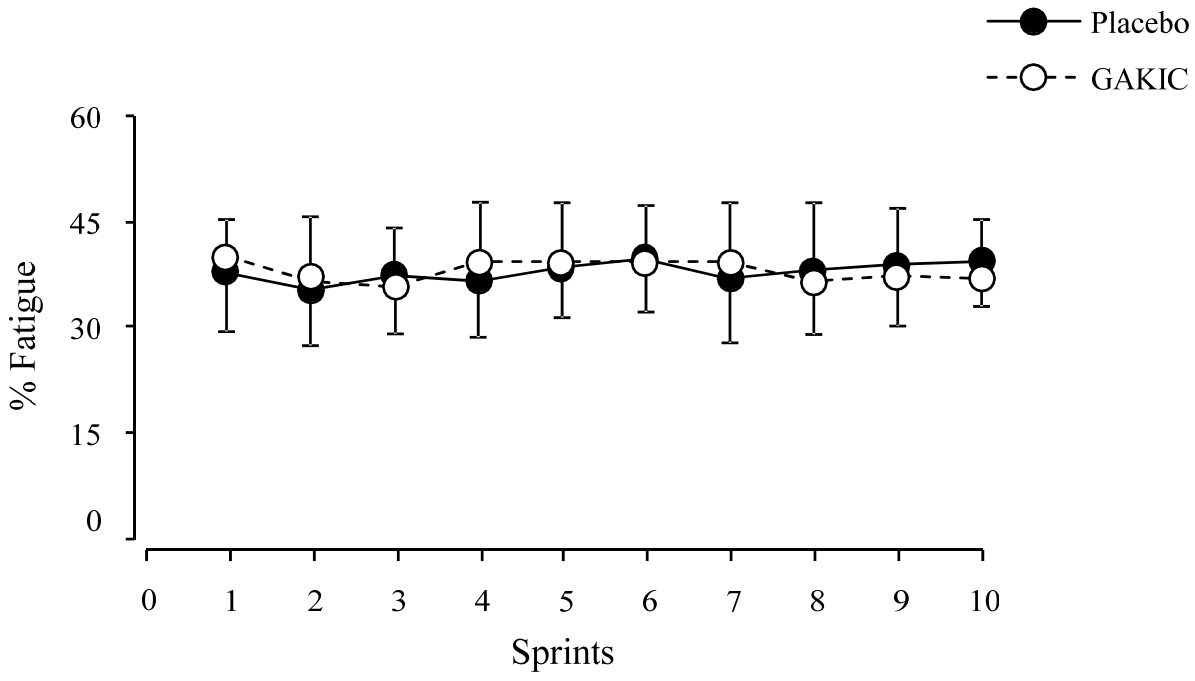


Figure 2.4 Fatigue index over ten repeated cycling sprints with GAKIC and Pl treatments (N = 10, mean ± SD).

Table 2.1 Comparison of Total Energy Intake, Macronutrients and Specific Amino Acids (mean ± SD).

Variable	GAKIC	Placebo	*P*
Energy (KJ)	11246 ± 3084	10629 ± 3808	0.5
CHO (g)	392 ± 110	406 ± 143	0.7
Protein (g)	98 ± 38	90 ± 39	0.3
Fat (g)	86 ± 44	66 ± 44	0.6
Arginine (mg)	1727 ± 1158	1400 ± 585	0.3
Leucine (mg)	2358 ± 1755	2496 ± 1602	0.8
Glycine (mg)	1393 ± 925	1360 ± 1023	0.9

The RPE increased from the 1st sprint (Pl: 13 ± 3, GAKIC: 13 ± 3) to the 10th sprint (Pl: 19 ± 1, GAKIC: 20 ± 1) and did not differ between treatments ($P = 0.11$). Additionally, HR increased from the first 1st (Pl: 151 ± 10, GAKIC 152 ± 12 beats·min^{-1}) to the 10th sprint (Pl: 166 ± 11, GAKIC: 164 ± 12 beats·min^{-1}) and did not differ between conditions ($P = 0.83$).

2.3.1 Side Effects

Generally, all subjects tolerated the supplementation protocol well, with no reports of gastrointestinal distress.

2.4 Discussion

The findings of the present study suggest that GAKIC supplementation does not have an ergogenic effect on muscle power output or attenuation of fatigue during repeated sprints in trained cyclists when administrated during a 45-min period prior the exercise. These results are in contrast to our initial hypothesis developed from previous literature which suggested that supplementation with GAKIC would enhance peak power output and attenuate the decline in power output during repeated high intensity sprints. In the present

study (Chapter 2), GAKIC had no effect on peak power, mean power, minimum power or fatigue index. Additionally, no differences were found in HR or RPE between the two experimental conditions.

The data reported in the present study contradicts both previous performance studies where GAKIC was found to attenuate the decline in power output, improve muscle performance (Buford & Koch, 2004) and delay muscle fatigue resulting in improving total work (Stevens, et al., 2000) during high intensity exercise. In the initial study, (Stevens, et al., 2000), demonstrated that GAKIC supplementation increased muscle force production by up to 28%, increased total muscle work by at least 12% and improved overall performance compared to Pl. The high intensity experimental protocol of Stevens, et al., (2000), consisted of 35 maximal, isokinetic repetitions at a rate of $90°\cdot s^{-1}$ knee extensions. In the later study, Buford & Koch (2004) reported a significant difference in mean power output during high intensity exercise consisting of 5 repeated sprints of 10 s on a cycle ergometer. On the other hand, a more recent study (Yarrow, Parr, White, Borsa, & Stevens, 2007), failed to find any ergogenic effect during moderate or high intensity exercise when the -α-ketoisocaproic acid (KIC) component of GAKIC was examined in isolation.

In both previous studies (Buford & Koch, 2004; Stevens, et al., 2000), it was hypothesised that the specific combination of amino- and keto-acids contained in GAKIC could work synergistically to improve high intensity exercise performance through their relevant metabolic pathway(s). In theory this hypothesis could be proposed as the individual components of GAKIC have been shown to contribute to the detoxification of ammonia (Greenstein, et al., 1956; Meneguello, Mendonca, Lancha, & Costa Rosa, 2003) which is released from the purine nucleotide cycle (Meyer & Terjung, 1979; Terjung, et al., 1985) during high intensity exercise or from the catabolism of protein and deamination of amino acids (Sitren & Fisher, 1977; van Hall, van der Vusse, Soderlund, & Wagenmakers, 1995). The detoxification of ammonia could theoretically enhance high intensity exercise performance as ammonia accumulation has been shown to be linked to muscular fatigue (Wilkinson et al., 2010), although the precise mechanisms remain to be determined. However, when the effect of each GAKIC component on exercise performance was examined (Bower et al., 1995; Daly et al., 1988; Jeevanandam, et al., 1993; Nair, et al., 1992; Weimann et al., 1998) there was little evidence to support an ergogenic effect.

Arginine appears to be essential for human metabolism only under specific circumstances such us burns, trauma, cancer (Bower, et al., 1995; Daly, et al., 1988; Weimann, et al., 1998) and other conditions of rapid growth apparently via the production of a number of hormones such as growth hormone (Besset, Bonardet, Rondouin, Descomps, & Passouant, 1982). However, the majority of studies using a variety of doses of L-arginine have failed to show any effect on altering nitric oxide production (Liu et al., 2009) or influencing hormone levels (e.g., growth hormone) or indeed exercise performance (Liu, et al., 2009; Marcell et al., 1999; McConell, Huynh, Lee-Young, Canny, & Wadley, 2006). Additionally, large doses of arginine have been shown to cause gastrointestinal discomfort (unpublished data cited in (Wagenmakers, 1999). On the other hand, glycine has an active role in the formation of Cr phosphate (as one of the three amino acids involved in synthesis of Cr), which is the main source of energy during intense exercise, such as employed in the present study. The synthesis of Cr from arginine and glycine is dependant on the formation of guanidinoacetate which is formed by the enzyme arginine:glycine amino-transferase (AGAT) (Walker, 1979). Excess Cr leads to downregulation of AGAT and in doing so inhibits the formation of guanidinoacetate (Stead, Au, Jacobs, Brosnan, & Brosnan, 2001). Therefore, the rate limiting step in the formation of Cr is not the concentration of arginine but the formation of guanidinoacetate from AGAT (Campbell, et al., 2004). Consequently, supplementation with arginine would not necessarily lead to a higher intramuscular Cr concentration. Previous studies involving KIC or Leucine supplementation (individually or in conjunction with other amino acids such as the branched-chain amino acids) have also failed to find any effect on aerobic or indeed anaerobic performance (Madsen, et al., 1996; Pitkanen, et al., 2003; van Hall, Raaymakers, et al., 1995; Yarrow, et al., 2007). Leucine supplementation seems ineffective in altering nitrogen balance (Sandstedt, Jorfeldt, & Larsson, 1992), protein degradation (Sandstedt, et al., 1992) or reducing the concentration of ammonia (MacLean, Graham, & Saltin, 1996; Madsen, et al., 1996). Therefore, the underlying mechanism behind the performance enhancing effects of GAKIC supplementation reported in the two aforementioned studies (Buford & Koch, 2004; Stevens, et al., 2000) remains elusive.

Alternatively and possibly most likely, the findings in the previous studies (Buford & Koch, 2004; Stevens, et al., 2000) could be due to the experimental design and methodology employed. For example, (Stevens, et al., 2000), utilizing untrained subjects failed to include baseline trials to establish the repeatability of the performance trials. It remains a distinct possibility that the differences in power output observed in the previous

studies (Buford & Koch, 2004; Stevens, et al., 2000) were due to a lack of reliability between performance trials, especially since non-trained subjects were utilized (Hopkins, et al., 2001; Coleman, Wiles, Nunn, & Smith, 2005; Hebestreit, Dunstheimer, Staschen, & Strassburg, 1999). Although $\dot{V}O_2$max of the subjects presented in this Chapter was not measured, peak power output is an established good predictor of $\dot{V}O_2$max (Hawley & Noakes, 1992). As such, peak power output of the first sprint in the present study was 1297 ± 263 W for the Pl trial and 1353 ± 283 W for the GAKIC trial as compared to approximately 750 W in the study by Buford & Koch (2004) reflecting the clear difference is training status of the subjects between studies. Therefore, there are several possible explanations for the difference in reliability between athletes (used in the present study) and non-athletes (used in the previous studies by Stevens, et al., 2001 and Buford, et al., 2004). Firstly, the frequent exposure to high-intensity exercise on an almost daily basis may eliminate performance variability (Hopkins, et al., 2001). Secondly, physical conditioning during the course of a study should not change to the same extent in trained as in non-trained individuals. Thirdly and possibly the most likely explanation is the custom to detail approach typically associated with trained cyclists (e.g., using customised cycling shoes and pedals, fixed cycling position etc.). Finally, assuming a constant random error contributed by equipment or examiners, this error will have less impact on overall outcome when expressed as a percent of the higher power output of trained individuals (Hopkins, et al., 2001). In the present study, variability in performance trials between familiarization trials was small (i.e., less than 5%) as would be expected from subjects who are well accustomed to the exercise protocols employed (Hopkins, et al., 2001; Coleman, et al., 2005; Hebestreit, et al., 1999). The more stringent methodology used in the present study ensured that a number of possible confounding factors were minimised. However, it should be noted that no attempt was made to verify the presence of the supplement active ingredients which is clearly a limitation of the present study (Chapter 2). Furthermore, the study design was not focussed on the null hypothesis and as a consequence it is lacking power to eliminate it. Future studies should be designed to ensure that the null hypothesis can be rejected with confidence.

2.5 Conclusion

The data presented in this Chapter do not support the findings of previous studies (Buford & Koch, 2004; Stevens, et al., 2000) that GAKIC supplementation will enhance high intensity exercise performance. After controlling for all possible confounding factors that

could have adversely affected the results, GAKIC supplementation had no effect on peak power, mean power or fatigue index. On the basis of this finding and those of previous studies involving the individual components of GAKIC, there appears little evidence to support the ergogenic effects of GAKIC during high intensity exercise performance when applied to well-trained subjects.

3. Food and macronutrient intake of elite Ethiopian distance runners

3.1 Introduction

The IAAF Consensus Statement on Nutrition for athletics published in 2007 states: "Well chosen foods will help athletes train hard, reduce risk of illness and injury, and achieve performance goals, regardless of the diversity of events, environments, nationality and level of competitors." (IAAF, 2007). Specific nutritional recommendations for optimal performance, particularly for endurance athletes, include a daily CHO intake ranging from 6 to 10 $g \cdot kg^{-1}$ BM considered essential for replacing liver and muscle glycogen stores (Rodriguez, et al., 2009). A significant protein intake ranging between 1.2 to 1.7 $g \cdot kg^{-1}$ BM per day is required for optimal health and performance of endurance athletes (Rodriguez, et al., 2009). Studies examining protein intake in athletes have shown an increased requirement for protein in endurance trained athletes (Friedman & Lemon, 1989; Gaine et al., 2006; Meredith, Zackin, Frontera, & Evans, 1989) as opposed to healthy adult males (i.e., 0.8 $g \cdot kg^{-1}$) due to increased amino acid oxidation during exercise and for growth and repair of muscle tissue (Genton, 2011). Maintenance of normal body water during strenuous training and minimising the level of dehydration (i.e., preventing a BM loss of > 2-3%) during endurance exercise achieved by consuming fluids at a rate of 0.4 to 0.8 $L \cdot h^{-1}$ *ad libitum* is now recommended (Sawka, et al., 2007). More studies that employ truly world class athletes are required in order to assess and possibly improve the applicability of the current recommendations to elite athletes. Therefore, there is an urgent need for novel data that can be obtained from some of the best athletes in the world.

Ever since Abebe Bekele became the first black African gold medallist in winning the marathon at the Rome Olympics in 1960, scientists have tried to explain the phenomenal success of east African distance runners in international athletics (Fudge et al., 2008; Onywera, Kiplamai, Boit, & Pitsiladis, 2004; Scott, et al., 2009; Scott & Pitsiladis, 2007). Notably, middle- and long-distance runners from Ethiopia and Kenya hold over 90% of both all-time world records as well as the current top-10 positions in world event rankings (IAAF, 2008). Possible explanations have been proposed including genetic factors (Hamilton, 2000; Scott et al., 2003), environmental conditions (Onywera, et al., 2004; Onywera, Scott, Boit, & Pitsiladis, 2006) and near optimal dietary practices (Christensen, Van Hall, & Hambraeus, 2002; Mukeshi & Thairu, 1993; Onywera, et al., 2004).

However, the east African running phenomenon still remains largely unexplained. While a significant number of studies have investigated putative factors influencing the east African running phenomenon, only five studies have assessed the dietary practices of elite east African runners and all have involved Kenyan athletes (Christensen, et al., 2002; Fudge, et al., 2008; Fudge et al., 2006b; Mukeshi & Thairu, 1993; Onywera, et al., 2004). The first of these studies, Mukeshi and Thairu (Mukeshi & Thairu, 1993) estimated the EI of male, long distance Kenyan runners through a combination of questionnaires and direct observation. Remarkably low EI (9790 $kJ \cdot day^{-1}$ on average) was reported, while the average CHO intake was 441 g (8.1 $g \cdot kg^{-1}$ BM per day) or 75% TEI. However, in the subsequent studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004), substantially higher estimates of EI were noted in comparison to the initial data. For example, Christensen et al. (2002) reported an average EI of 13210 $kJ \cdot day^{-1}$, while the consumption of CHO was 476 g (8.7 $g \cdot kg^{-1}$ BM, 71 % of TEI). Similarly, Onywera et al. (2004) reported an average EI of 12486 $kJ \cdot day^{-1}$ (CHO 607 g, 10.4 g/kg BM and 76.5% TEI), while estimated EI in two studies by Fudge and colleagues were 13241 $kJ \cdot day^{-1}$ (CHO 552 g, 9.8 $g \cdot kg^{-1}$ BM and 71% TEI) (Fudge, et al., 2006b) and 12300 $kJ \cdot day^{-1}$ (CHO 580 g, 9.8 $g \cdot kg^{-1}$ BM, 79% TEI) (Fudge, et al., 2008), respectively. These dietary studies focused primarily on athletes from the Kalenjin tribe of Kenya; a fairly distinct Kenyan ethnic group living at high altitudes, noted for producing athletes of great endurance. For example, the Kalenjin tribe has less than 0.1% of the world's population, yet members of this tribe have achieved nearly 50 athletic Olympic medals.

Ethiopian athletes boast a recent success record in international distance running second only to Kenya. As is the case in Kenya, successful Ethiopian athletes come predominantly from one localized ethnic group in the Ethiopian region of Arsi (Scott, et al., 2003). The Arsi region of Ethiopia is situated at high altitude and contains roughly 5% of the Ethiopian population whilst accounting for 14 of the 23 distance runners selected for the country's 2008 Olympic team. Historically there has been very little cultural exchange or indeed biological intermingling between Kenyans (Scott, et al., 2009) and Ethiopians, therefore very distinct dietary habits can be found in the two countries, even between tribes within each country. Consequently and given the absence of dietary data for Ethiopian athletes, the main aim of the investigation presented in Chapter 3 was to assess the dietary practices of elite Ethiopian endurance runners to elite Kenyan athletes during an important training period, as well as to the current recommendations for endurance athletes. This

investigation also aimed to provide a rare insight into the lifestyle and training practices of some of the most successful endurance runners in the world prior to major competitions.

3.2 Methods

3.2.1 Subjects

Ten highly-trained (8 male, 2 female) Ethiopian distance runners gave their written informed consent to take part in the present study which was approved by the local ethics committee (Research Ethics Committee, Department of Physical Education and Sport Science, Addis Ababa University, Addis Ababa, Ethiopia) and was performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). Subjects were highly trained (best marathon time: 2:13:55 ± 0:01:42; mean ± SD; Table 3.1) and in excellent condition (trained twice daily) while preparing for major competitions (e.g., 2008 Beijing Olympic Games, 2008 Berlin marathon). Athletes recruited were managed by Global Sports Communication (http://www.globalsportscommunication.nl/); arguably the most accomplished of all the track and field athlete management organizations specializing in middle- and long-distance running events. Athletes living and training at the training camp under the management of Global Sports Communication all follow very similar training practices. Athletes residing at the Global training camp included world record holders, medallists at major championships such as the Olympic Games, World Championships and major city marathons like the London Marathon. The present study was conducted during the period when some of the athletes were preparing for the 2008 Beijing Olympics. The physical characteristics of the athletes included in the present study were measured according to the 2006 ISAK procedures (Marfell, 2006) and are presented in Table 3.1.

Table 3.1 Physical characteristics of the Ethiopian runners.

Subject (no)	Age (y)	Height (m)	Start BM (kg)	End BM (kg)	Change BM (%)	Change BM (kg)	BT (M)	BT (F)
1	23	1.72	58.7	58.7	0.0	0.0	2:12:00	
2	21	1.78	62.4	61.5	1.4	-0.9	2:12:00	
3	22	1.72	59.8	59.9	-0.1	0.1	2:13:15	
4(F)	19	1.75	57.3	57.4	-0.2	0.1		2:35:03
5(F)	19	1.61	48.8	48.3	1.0	-0.5		2:30:15
6	23	1.73	57.7	58.5	-1.4	0.8	2:15:15	
7	27	1.81	53.5	53.3	0.4	-0.2	2:14:10	
8	20	1.76	61.7	61.0	1.1	-0.7	2:12:35	
9	23	1.73	53.4	53.6	-0.4	0.2	2:15:45	
10	23	1.65	53.3	53.4	-0.2	0.1	2:16:17	
Average	22	1.73	56.7	56.6	0.2	-0.1	2:13:56	
SD	2	0.06	4.3	4.2	0.8	0.5	0:01:42	

* *Note:* M, male; F, female; BM, body mass; BT, best marathon time.

3.2.2. Experimental Procedures

The dietary intake of the athletes was assessed in the month of July (ambient temperature: 12 to 21 ^{o}C) during a period of intense training prior to major competition using 7-day weighed dietary records (Lissner, Heitmann, & Lindroos, 1998). The dietary intake of the athletes was directly observed, weighted and recorded. All athletes competed in endurance running events ranging from 10 km to the marathon and lived in a single training camp (Global Sports training camp Addis Ababa - Kotebe, 8° 58' 0 N, 38° 49' 60 E) which was based at high altitude (~2400 m above sea level). During the 7 days, subjects followed their habitual eating/drinking pattern, as was confirmed by the manager/coach of the training camp. Training was assessed using a training diary which included the type, intensity and duration of exercise training. The training diary, in combination with direct observation, was used to estimate energy expenditure (EE) (Table 3.2). Briefly, total EE was estimated from the duration and intensity of each activity, using physical activity ratios (PAR) (Ainsworth et al., 2000). The energy cost was expressed as a multiple of basal metabolic rate (BMR). In the current study, BMR was estimated using the Schofield equations (Schofield, 1985). It should be noted that the training intensity and EE data has been generated in the present study using indirect methods (Ainsworth, et al., 2000). Nevertheless, the results of these indirect methods are reported in order for the results of the current study to be directly comparable to the data generated in previous studies using similar methods (Onywera, et al., 2004).

Table 3.2 Estimated daily energy expenditure according to Physical Activity Ratio.

		Duration (h)		Energy cost (PAR)	
	PAR[a]	MEAN	SD	MEAN	SD
Sleeping	0.9	9.0	0.8	8.1	0.7
Relaxing[b]	1.0	5.7	0.5	5.7	0.5
Miscellaneous activity[c]	1.5	6.7	0.0	10.1	0.0
Light exercise[d] (home activities)	3.0	0.5	0.1	1.4	0.2
Slow pace running	10.0	0.1	0.2	1.5	1.6
Moderate pace running	14.0	0.9	0.3	13.1	3.7
Fast pace running	18.0	0.7	0.2	12.2	4.0
Total		24	0.6	52.1	3.3

**Note:* SD, standard deviation; [a]Physical activity ratio (PAR) is the energy expenditure expressed in relation to basal metabolic rate (BMR) (i.e., BMR × 1.0); [b]Watching TV, sitting quietly; [c]Eating, socializing; [d]Home activities.

The subjects weighed and recorded all food and drink consumed using individual kitchen weighing scales accurate to 1 g (Salter Housewares LTD, England). All food was weighed before and after cooking. The cooking method was also described and recorded. Athletes self-selected their portion sizes from the food provided *ad libitum*. The remaining food (leftover) was also weighed and deducted from the initial weight of portion. The participants were also required to use the weighing scales when they were away from the training camp and to disclose any extra food intake during the hours when direct observation was not possible. Details on how to report food and fluids consumed were given to each subject in English and in their local dialect (i.e., Oromo or Amharic). Total water intake was assessed by combining the reported dietary intake of water with the estimated metabolic water value as previously described and conducted in elite Kenyan athletes (Fudge, et al., 2008; Fudge, et al., 2006b). Metabolic water was determined by multiplying the measured EE by the fraction of energy in the diet obtained from CHO, protein and fat (data derived from the 7-day nutritional records). This value was then multiplied by water obtained from CHO, protein and fat oxidation (0.60, 0.41 and 1.07 mL water$\cdot g^{-1}$, respectively) (Fjeld, Brown, & Schoeller, 1988). To improve the quality of the collected data and to avoid any problems or under reporting of food or fluids consumed, one of the researchers resided at the camp for the entire assessment/observational period. Meals were prepared whilst athletes trained and served at the same times every day: Breakfast was at 09:30, after the morning training session, lunch at 13:30 and dinner at 19:30. On some occasions, athletes also had an afternoon snack which was served at 16:00. Nude BM was measured on the first day of the assessment period (as well as for two days prior to the start of the assessment period to ensure a representative baseline) and at the end of the 7-day period, before the consumption of any food or drink. The weighed

dietary intake data were used to determine EI and diet composition using a computerised version of the food composition tables of McCance and Widdowson as revised by Holland et al. (1991). However, for foods more specifically consumed by Ethiopians, food tables published by the Ethiopian Ministry of Health of Ethiopia were used (Ethiopian Health and Nutrition Research Institute, 1998). No samples were retained for further analysis due to local regulations. Food labels were also collected where possible, mainly for imported foods. Illustrations of the lab work are presented in Figure 3.1.

Figure 3.1 Illustrations of the field work data collection.

3.2.3 Data Analysis

Data were expressed as the mean ± SD, as appropriate following a test for the normality of distribution. Paired t-tests were used to compare EI vs. EE and starting BM vs. final BM. Statistical significance was declared when $P < 0.05$. All statistical analysis was completed using the software package SPSS, version 15.0 (SPSS, Inc., Chicago, IL, USA).

3.3 Results

Training typically consisted of two sessions per day. The morning run (normally at 07.00) took place before breakfast and included a session at moderate or fast pace (16-20 km·h^{-1}) for 10 to 20 km depending on the instructions given by the coach and/or weather conditions. The afternoon session, prior to dinner (17.00), was typically an easy run over 6 to 10 km at a slower pace (10-15 km·h^{-1}), unless morning weather conditions had been adverse. If this was the case, athletes reversed their sessions. Warming up periods were 15 min and cooling down periods were more than 20 min. Warm up and cool down consisted of standard stretching exercises and athletes carried out most of their sessions as a group. In some instances, some athletes trained alone. Athletes completed high intensity interval training sessions 2-3 times per week and one 20-25 km run at near race speed for each athlete. Recovery time between training sessions was spent at the camp sleeping, eating, socialising, watching television or washing their clothes. Some athletes went home on weekends and completed individual training runs as advised by their coach/manager. The EE of the athletes as estimated using PAR is shown in Table 3.2.

Estimated EI over the 7-day assessment period (13375 ± 1378 kJ) was matched by estimated EE (13670 ± 862 kJ; P = 0.69). BMR was estimated at 6292 ± 565 kJ·day^{-1}. The BM remained stable over the 3 days prior the assessment period (pre: 56.6 ± 4.1 kg vs. post: 56.7 ± 4.3 kg; P = 0.58). The athletes' BM (pre: 56.7 ± 4.3 kg vs. post: 56.6 ± 4.2 kg; P = 0.54) remained stable over the 7 days (Table 3.1). The diet consisted mainly of vegetable sources (approximately 88%) with only a small portion of meat (approximately 12%; Table 3.3). Breakfast consisted typically of milk, porridge, omelette and bread. Lunch comprised mainly of vegetable sources such as pasta, rice and lentils, while meat was served only twice a week and dinner was similar to lunch. Food portions were chosen by the subjects themselves (i.e., *ad libitum*), as no advice or guidelines were given. Furthermore, two of the athletes consumed commercially available nutritional supplements (i.e., 100 g of the supplement consisted of 95.1 g CHO of which sugars 59.7 g, L-Glutamine 250 mg, L-Leucine 110 g, L-Valine 100 g, L-Isoleucine 70 mg, and Sodium 0.9 g). As for fluid intake, subjects consumed water with modest amounts of tea, milk, orange juice and a local drink called Besso, a mixture of barley and water. The diet was high in CHO intake (64.3 ± 2.6% TEI, 545 ± 49 g, 9.7 ± 0.9 g·kg^{-1} BM per day (Figure 3.2, Figure 3.3). The fat intake of the diet was 23.3 ± 2.1% TEI and 83 ± 14 g daily (Figure 3.2, Figure 3.3). Protein intake was 12.4 ± 0.6% TEI, 1.8 ± 0.2 g·kg^{-1} and 99 ± 13 g per day

(Figure 3.2, Figure 3.3) of which 76% was derived from vegetable sources (Table 3.3). Daily fluid intake consisted mainly of water (1751 ± 583 $mL \cdot day^{-1}$; 55.4% of the total water intake), while the athletes did not consume any fluids before or during their training sessions. Other sources of daily fluid intake were water consumed as moisture in food (950 ± 60 mL; 29.9%) and metabolic water produced as a result of the oxidation of CHO, protein, and fat (470 ± 28 mL; 14.8%) which resulted in a mean total daily fluid intake of 3.2 ±0.6 $L \cdot day^{-1}$.

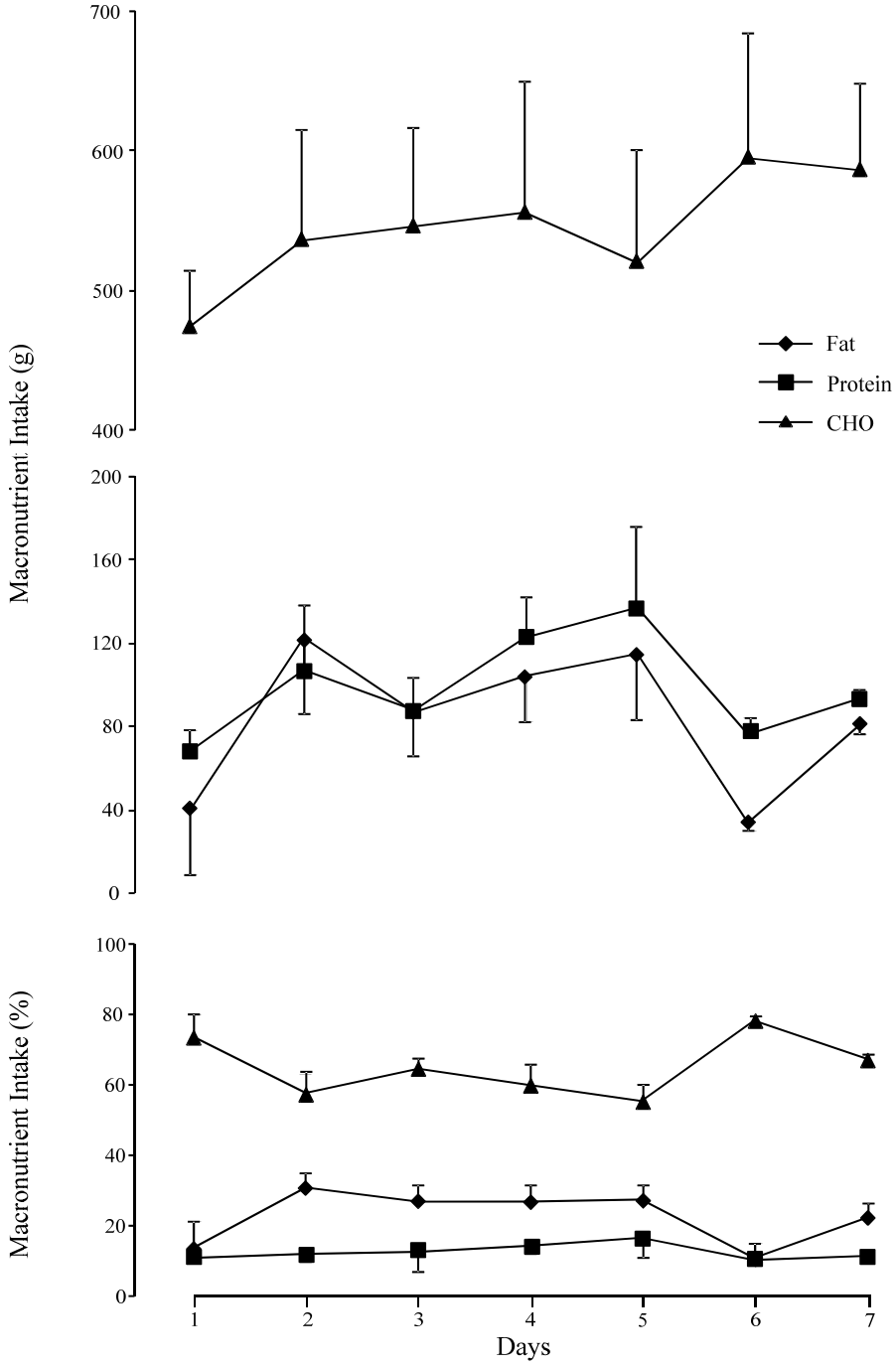


Figure 3.2 Macronutrient intake (g and percent intake) (mean ± SD) over the 7 day period.

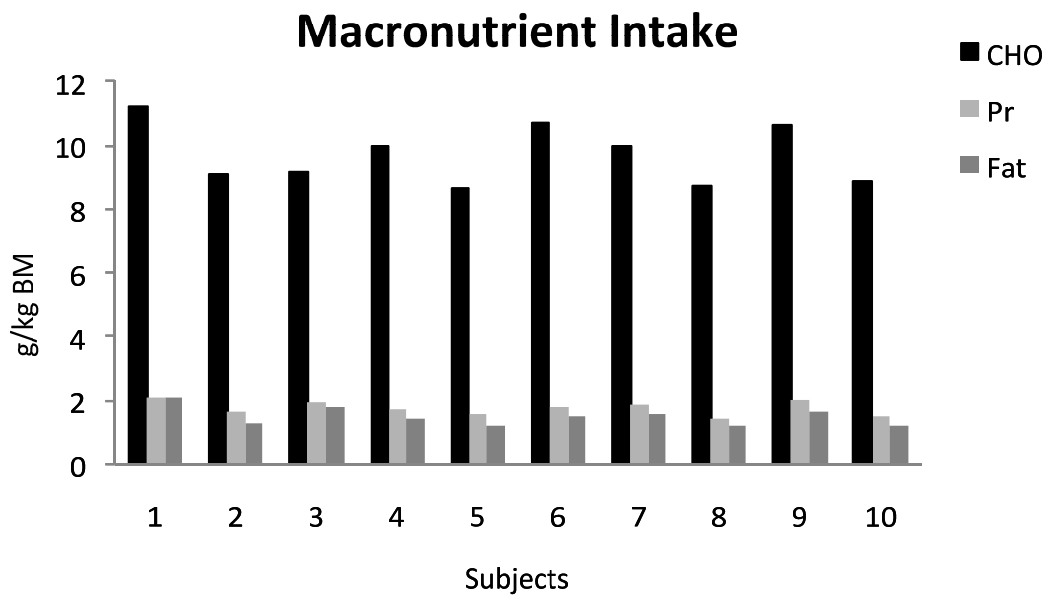


Figure 3.3 Individual ranges of macronutrient intake (average for the 7-day period).

Table 3.3 Food Sources as a percentage of daily intake of each macronutrient.

Food Sources (%)	Energy (kJ)	CHO (g)	Fat (g)	Protein (g)
Porridge	4.5	5.5	2.1	3.0
Bread	15.2	18.7	4.7	17.5
Pasta	10.0	12.0	3.1	13.4
Rice	5.0	6.5	1.8	2.8
Injera	20.8	27.3	4.8	16.5
Meat	5.3	0.1	16.1	11.9
Lentils	2.4	1.8	3.6	3.5
Sugar[a]	3.5	5.4	0.0	0.0
Eggs	1.5	0.1	3.9	4.0
Milk	1.3	0.6	3.1	2.1
Vegetable Oil	10.2	0.0	43.5	0.0
Chick Peas	1.0	0.9	0.6	1.9
Shiro	2.1	1.5	2.4	4.7
Total	83	85	90	84
Other[b]	17	15	10	16
Animal source	12	1	27	24
Vegetable source	88	99	73	76
Mean	3194	545	83	99
SD	329	49	14	13

[*]*Note.* SD, standard deviation; [a]Sugar consumed in tea and porridge; [b]Food sources that contribute less than 1%.

3.4 Discussion

The findings of the study present in Chapter 3 indicate that Ethiopian endurance runners whilst meeting dietary recommendations for endurance athletes for macronutrient intake did not do likewise as far as fluid intake was concerned. In the present study (Chapter 3), the dietary intake data were used to estimate the EI, while the EE and BM data were interpreted in the context of energy balance and in order to assess under eating. Total average EI was 13375 ± 1378 kJ and is in agreement with previous studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004) (~ 12809 kJ·day^{-1} on average). In the first of these studies conducted in Kenyan athletes, Mukeshi and Thairu (Mukeshi & Thairu, 1993) estimated the EI of male, long distance Kenyan runners through a combination of questionnaires and direct observation and reported remarkably low EI (9790 kJ·day^{-1} on average). However, in subsequent studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004), substantially higher estimates of EI were reported in comparison to the initial data. For example, Christensen

et al. (2002) reported an average EI of 13210 kJ·day^{-1}. Similarly, Onywera et al. (2004) reported an average EI of 12486 kJ·day^{-1}, while estimated EI in two studies by Fudge and colleagues were 13241 kJ·day^{-1} (Fudge, et al., 2006b) and 12300 kJ·day^{-1} (Fudge, et al., 2008). A finding common to most of the aforementioned studies was the lower EI compared to EE and therefore indicative of negative energy balance before major competition (Fudge, et al., 2006b; Onywera, et al., 2004). It is well acknowledged that training at high altitude can impact negatively on energy balance (Westerterp-Plantenga, Rolland, Wilson, & Westerterp, 1999), most likely due to a reduction in EI brought about by a loss of appetite (Ward, 1995). However, in contrast to previous studies in Kenyan runners (Fudge, et al., 2006b; Onywera, et al., 2004), Ethiopian runners recruited in this study met their energy needs (EI did not differ from EE) and consequently maintained their BM (pre assessment period BM: 56.7 ± 4.3 kg vs. post: 56.6 ± 4.2 kg). This is consistent with recent guidelines by the ACSM that advocate that differences between EI and EE could compromise performance and negate the benefits of training (Rodriguez, et al., 2009).

Macronutrient intake of Ethiopian long distance runners fulfilled recent recommendations (Rodriguez, et al., 2009). CHO intake was 64.3% TEI (9.7 g·kg^{-1} BM per day) and the daily CHO intake was 545 ± 49 g (Figure 3.2), while recommendations for male and female athletes range between 6 to 10 g·kg^{-1} BM per day (Rodriguez, et al., 2009). These results are also in agreement with previous studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Mukeshi & Thairu, 1993; Onywera, et al., 2004) when the daily amount of CHO was well above 65% of TEI, ranging from 8.1 to 10.4 g·kg^{-1} BM and within the current recommendations (Rodriguez, et al., 2009). Protein intake was 12.4% of TEI (Figure 3.2) (1.76 g·kg^{-1} BM per day with a daily intake of 99 ± 13 g) of which 76% was delivered from vegetable sources (Table 3.3) and well within the current recommendations for endurance athletes (1.2 to 1.7 g·kg^{-1} BM per day) (Rodriguez, et al., 2009). This is also in agreement with the literature (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004) where daily protein intake ranged from 1.3 to 2.2 g·kg^{-1} BM. Adequate protein and fat intake are also vital for optimal health and performance of long distance runners. Sufficient dietary protein will provide essential amino acids and maintain the nitrogen balance for building and repair of muscle tissue after intense endurance training (Rodriguez, et al., 2009). Furthermore, having achieved the recommended amounts of CHO and protein, this would have resulted in a sufficiently high intake of fat to ensure an important source of fat soluble vitamins and essential fatty

acids (Coyle, Jeukendrup, Oseto, Hodgkinson, & Zderic, 2001; Rodriguez, et al., 2009). Hence, the fat intake of distance runners especially from developing countries should not be restricted further as there would be no performance benefit in consuming less fat than that observed in the current study (23.3% TEI). Rodriguez et al. (2009) reported that there are no advantages in consuming a diet with less than 15% of energy from fat compared with 20 to 25% of TEI. Although the values from the present study (23.3% TEI, Figure 3.2) for fat intake are in agreement with the guidelines (Rodriguez, et al., 2009), they were somewhat higher in comparison to values (6.6 to 17.4% of TEI) observed in previous studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Mukeshi & Thairu, 1993; Onywera, et al., 2004). Moreover, the fact that vegetable sources accounted for approximately 88% of TEI (Table 3.3) concurs with other published dietary studies for low income countries (Cerqueira, Fry, & Connor, 1979; Christensen, et al., 2002; Mukeshi & Thairu, 1993) and contrasts with that for developed countries (Burke, Gollan, & Read, 1991; Grandjean, 1989; van Erp-Baart, Saris, Binkhorst, Vos, & Elvers, 1989). For example, the CHO intake of elite distance runners in the United States (Grandjean, 1989), the Netherlands (van Erp-Baart, et al., 1989) and Australia (Burke, et al., 1991) was 49%, 50% and 52% of TEI respectively, as a result of a more varied diet.

Optimizing fluid replenishment is fundamental during exercise. Correct fluid replacement practices are especially crucial in endurance events lasting longer than an hour where the participating athlete might have not consumed adequate food or fluid before exercise or in cases where the athlete is exercising in an extreme environment (heat, cold, or high altitude) (Rodriguez, et al., 2009). It is perhaps surprising that in the present study (Chapter 3), the Ethiopian endurance athletes taking part in prolonged intense exercise and/or extreme conditions, did not fulfil the current recommendations for fluid intake (Sawka, et al., 2007). In fact, the athletes consumed approximately 1.75 $L \cdot day^{-1}$ of fluids which comprised mainly of water and athletes in general did not consume water before or during training; on some occasions small amounts of water was consumed following training. This finding is in line with previous findings (Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004). Onywera and colleagues (2004) reported a modest fluid consumption (2.3 $L \cdot day^{-1}$). Additionally, similar fluid intake (1.8 $L \cdot day^{-1}$) was observed by Fudge et al. (2006) and in a subsequent study by the same group (2.3 $L \cdot day^{-1}$) (Fudge, et al., 2008). These studies collectively show that these elite athletes do not consume any fluids before or during training, while modest amounts of fluids are consumed after training and only by a small number of runners (Fudge, et al., 2008; Fudge, et al., 2006b;

Onywera, et al., 2004). According to current recommendations, the amounts of fluid consumed (as dietary water intake) in the present study would be inadequate to maintain athletes' hydration status (Sawka, et al., 2007). Nevertheless, when total water intake (i.e., dietary water intake and metabolic water) is considered, Ethiopian athletes are found to be well hydrated during the day due to the high quantity of water in their staple foods (e.g., injera). Furthermore, although fluid consumption in the present study was less than recommended (Sawka, et al., 2007), the daily total *ad libitum* water intake (0.23 ± 0.04 $L \cdot MJ^{-1}$) was consistent with guidelines from the US National Research Council (National Research Council, 1989). These guidelines suggest 1 mL of water per kcal (0.24 $L \cdot MJ^{-1}$) of EE for adults under average conditions of EE and environmental exposure with the rare exception of instructing the consumption of 1.5 $mL \cdot kcal^{-1}$ (0.36 $L \cdot MJ^{-1}$) in cases of higher levels of physical activity, sweating and solute load. Additionally, the total water intake in the current study (3.2 L) is in accordance with optimal kidney function and urine output maintenance at high altitude (i.e., 3–4 $L \cdot day^{-1}$) (Rodriguez, et al., 2009). This is also in agreement with the existing literature (Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004) where elite Kenyan distance runners maintained their hydration status due to the consumption of foods with a high quantity of water (e.g., ugali) (Onywera, et al., 2004). On the other hand, fluid intake recommendations as set by the ACSM guidelines indicate that athletes should consume 5-7 $mL \cdot kg^{-1}$ BM of fluids at least 4-h prior to the exercise session aiming to start the physical activity euhydrated with normal plasma electrolyte levels (Sawka, et al., 2007). Nevertheless, evidence to support this recommendation is equivocal at this point. It is important to note that mild dehydration may actually be an advantage as, theoretically, it will lower the energy cost of running at the same relative intensity (Armstrong, Costill, & Fink, 1985; Coyle, 2004).

3.5 Conclusions

As previously found in elite Kenyan endurance runners, elite Ethiopian runners met dietary recommendations for endurance athletes for macronutrient intake but not for fluid intake. Nevertheless, it remains unclear how these differences in dietary patterns with regard to fluid consumption, before major competitions, have an impact on their performance.

4. Drinking behaviours of elite male runners during marathon competition

4.1 Introduction

Fluid intake recommendations for endurance events such as the marathon have changed substantially over the last half century. During the first half of the twentieth century until the early 1970s, runners were typically advised to avoid ingesting fluid during competitive marathon running (Noakes, 1993). However, since the publication of the 1987 ACSM position stand on fluid intake, recommendations have typically advocated that all the sweat lost during exercise should be replaced by drinking the maximum amounts that can be tolerated (Binkley, et al., 2002; Convertino, et al., 1996; Montain, et al., 1999). More recently, the ACSM has replaced the 1996 Position Stand (Convertino, et al., 1996) with an updated version (Sawka, et al., 2007) that promotes drinking *ad libitum* (i.e., at one's pleasure) from 0.4 to 0.8 $L \cdot h^{-1}$ of fluid during exercise with lower fluid volumes for slower and lighter individuals competing in cooler environments, and higher volumes for faster and larger individuals competing in warmer environments. Athletes are also advised to drink enough to ensure that they do not lose more than 2-3% of BM, based on the premise that BM loss greater than 2% impairs exercise performance (Cheuvront & Haymes, 2001b; Coyle, 2004; Fudge, Pitsiladis, Kingsmore, Noakes, & Kayser, B. 2006; Sawka, et al., 2007). This advice also ensures that individuals do not gain BM since large increases in BM can cause hyponatraemia (i.e., serum sodium concentration <135 $mmol \cdot L^{-1}$) (Almond et al., 2005; Hew-Butler et al., 2008; Noakes et al., 2005).

We have previously shown that drinking *ad libitum* maintained hydration status day - to - day in elite Kenyan endurance runners (Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004). The value of proposing specific fluid intake ranges during exercise has been investigated by modelling parameters that influence sweat rate (Montain, et al., 2006). Montain et al. (Montain, et al., 2006) estimated that a fluid intake rate of 0.4 - 0.8 $L \cdot h^{-1}$ would maintain BM loss within 3% and prevent BM gain in 50 – 90 kg subjects running marathons at 8.5 – 15.0 $km \cdot h^{-1}$ in cool and warm ambient conditions (i.e., 18 ºC and 28 ºC, respectively). The authors suggested that factors that influence sweat rate such as BM, running speed and ambient conditions should also be considered before the recommended fluid intake range is adopted and that individual variation needs to be appropriately

considered (Montain, et al., 2006). However, the authors did not specifically consider the fluid requirements of elite marathon runners (Montain, et al., 2006). For example, maximum running speed used in these calculations was only 15 $km \cdot h^{-1}$, whereas to win a major city marathon, a male athlete must generally run faster than 19 $km \cdot h^{-1}$. Furthermore, the environmental temperatures (i.e., 18 ºC and 28 ºC) on the basis of which sweat rates and resultant BM loss were estimated in that analysis, do not adequately reflect the lower environmental temperatures typically experienced in many major city marathons (Cheuvront & Haymes, 2001b) or indeed the ambient conditions encountered when the world's fastest marathons are run (Fudge, et al., 2006a). For example, the top ten fastest marathons of all time (up to 2004) were performed at a mean ambient temperature of 7.3 ºC (Fudge, et al., 2006a). This observation is not unexpected given the strong evidence that exercise performance is strongly influenced by the prevailing environmental conditions (Galloway & Maughan, 1997).

Currently there are limited data on the drinking behaviours of the fastest marathon runners during competition (Cheuvront & Haymes, 2001b; Van Rooyen, et al., 2010). Therefore, in the present study, retrospective video analysis was used to determine the drinking behaviours of elite marathon runners competing in major city marathons. Determining the drinking behaviours of successful elite runners also allowed us to assess (albeit indirectly) the efficacy of current fluid intake recommendations (Sawka, et al., 2007) for this group of elite athletes. Furthermore, the sweat rates of 5 elite male distance runners (including 2 of the studied 10 marathon runners) were determined during training prior to the 2008 Beijing Olympic Games. According to the current drinking guidelines, a fluid intake rate is considered adequate if BM loss in athletes adopting that rate remains between 0 and 2-3% for the duration of the race (Cheuvront & Haymes, 2001b; Coyle, 2004; Fudge, et al., 2006a; Sawka, et al., 2007). Therefore, the efficacy of current fluid intake recommendations (Sawka, et al., 2007) for elite marathon runners is described in the study present in Chapter 4 by documenting the fluid intake behaviours of the given population.

4.2 Methods

4.2.1 Subjects and Experimental Procedures

A retrospective analysis of the drinking behaviours of 10 male athletes during 13 successful city marathons was undertaken using available video footage. Much of the

required footage was provided by the British Broadcasting Corporation. Permission for the present investigation was given by the respective race organizers. Elite runners participating in the laboratory/field studies gave their written informed consent to take part, approved by the local ethics committee and performed according to the code of ethics of the World Medical Association (Declaration of Helsinki).

Data were collected from the Athens Olympic Marathon 2004 and the Beijing Olympic Marathon 2008 as well as for the following city marathon races: Berlin 2006, 2008 and 2009, Chicago 2008 (1st and 2nd placed athletes), Dubai 2009, Fukuoka 2009, London 2006 and 2007, New York 2008 and 2009 and Rotterdam 2010. Only the winners of each race were targeted (unless otherwise stated), as the images were recorded from a motorcycle that followed the lead group(s). This methodological constraint resulted in maximum possible camera coverage of the race winners. Inevitably, however, there was no useful footage available for some drinking stations for various reasons such as the camera focusing on athletes others than those of interest. The observed duration of fluid consumption was measured from the available video footage. Where data from specific drinking tables was missing, the observed data for each athlete was extrapolated to provide information for the entire race (Table 4.1). For example, 6 of the 8 (75%) drinking stations were observed in the video footage of the Berlin 2006 marathon, so the data from the analysed footage were extrapolated to 100% (Table 4.2). Furthermore, the athletes competing in Beijing 2008, Chicago 2008, Chicago 2009 and New York marathons did not only use sport drink bottles (the maximal flow rates of which had been simulated in the laboratory), but they also used water bottles or cups from which flow rates are unknown. The percentage of the drinking episodes involving alternate container types is approximately 23%. For the runners of this study we had to assume a single flow rate from all these fluid dispensers. The video footage was retrospectively analyzed using Virtual Dub 1.6.12 Software (Copyright© 1998 - 2005 by Avery Lee) as this software allowed viewing of each individual frame. Drinking stations for elite runners were placed at or close to every 5 km of the course (as governing body regulations stipulate) and the time spent ingesting fluid (in seconds) was measured from the video images. It was assumed that drinking time occurred when the drinking bottle was raised above 90 º (horizontal) whilst in contact with the runner's lips. The entire race was also monitored for any additional fluid that may have been ingested along the route (e.g., general water and sports drink stalls). If there was no footage of the athlete of interest at a drinking station then an entry of no data (ND) was recorded. Fluid intake (mL) for each athlete was estimated by

multiplying the total duration spent ingesting fluid by 45.2 as this was the average flow rate ($mL \cdot s^{-1}$) obtained from a purposely designed laboratory drinking simulation. For this simulation experiment, the maximal flow rate (45.2 ± 13 $mL \cdot s^{-1}$) of a typical sports drink bottle was measured in 7 male volunteers who ingested as much fluid as possible within 3 s. This procedure was repeated 10 times whilst data were acquired from video footage which accurately measured the time taken to complete fluid ingestion. This was the same method used to determine drinking time from the video footage of the marathon runners. The estimated fluid intake rate (i.e., $L \cdot h^{-1}$) was compared to the current fluid replacement recommendations (Sawka, et al., 2007) which suggest that athletes should drink 0.4 – 0.8 $L \cdot h^{-1}$ of fluid *ad libitum*. A snapshot of the data collection process is presented in Figure 4.1.

Table 4.1 The temperature, relative humidity, finishing time, average pace, observed and extrapolated drinking time, estimated volume and rate for fluid intake of the athletes during 13 specified marathon races.

Marathon	Athlete (No)	Temp (^{o}C)	Humidity (%)	Time (h:mm:ss)	Pace ($km \cdot h^{-1}$)	Duration of drinking (Observed) (s)	Duration of drinking (Extrapolated) (s)	Total fluid intake (mL)	Extrapolated rate of fluid intake ($L \cdot h^{-1}$)
Athens 2004	1	30	39	2:10:55	19.35	3.7	11.2	506.2	0.23
Berlin 2006	2	20 ±3	30±13	2:04:26	20.29	21.6	28.8	1299.5	0.63
Berlin 2008	2	12±2	89±12	2:03:59	20.39	20.2	40.5	1829.7	0.89
Berlin 2009	2	16±1	72±8	2:06:08	20.00	17.4	24.3	1097.5	0.52
Dubai 2009	2	16±2	54±14	2:05:29	20.10	24.0	38.4	1735.7	0.83
Beijing 2008	3	27±2	52	2:06:32	20.00	12.4	19.6	885.9	0.42
Chicago 2008 1st	4	21±3	59±9	2:06:25	20.00	10.5	14.0	631.0	0.30
Chicago 2008 2nd	5	21±3	59±9	2:07:37	19.81	45.0	48.8	2204.9	1.04
Chicago 2009	3	0±1	70±5	2:05:41	20.10	10.5	12.0	542.4	0.26
London 2006	6	10±1	84±3	2:06:39	20.00	38.0	50.7	2289.8	1.09
London 2007	7	18±3	55±9	2:07:41	19.72	31.0	31.0	1401.2	0.66
Fukuoka 2009	8	7±1	63±7	2:06:10	20.00	20.5	32.3	1458.2	0.69
New York 2008	9	6±1	42	2:08:43	19.63	1.4	1.6	72.8	0.03
Rotterdam 2010	10	9±1	-	2:04:48	20.29	2.7	3.6	164.1	0.08

Table 4.2 The amount of time (s) the athlete was observed drinking at each feeding station during the different races.

Marathon	2.5km	5km	7.5km	10km	12.5km	15km	17.5km	20km	22.5km	25km	27.5km	30km	32.5km	35km	37.5km	40km	Total	Number of stations at which drinking measured (%)	Extrapolated total drinking time (s)
Athens 2004	NS	ND	ND	ND	ND	ND	ND	0.5	ND	0.0	ND	ND	0.0	ND	0.0	3.2	**3.7**	5/15 (33)	**11.2**
Berlin 2006	NS	0.6	NS	5.9	NS	2.3	NS	ND	NS	ND	NS	5.4	NS	4.8	NS	2.7	**21.6**	6/8 (75)	**28.8**
Berlin 2008	NS	7.2	NS	2.3	NS	8.7	NS	ND	NS	ND	NS	ND	NS	ND	NS	2.1	**20.2**	4/8 (50)	**40.5**
Berlin 2009	NS	3.6	NS	0.0	NS	ND	NS	1.8	NS	ND	NS	7.0	NS	5.0	NS	NS	**17.4**	5/7 (72)	**24.3**
Dubai 2009	NS	5.4	NS	ND	NS	5.8	NS	6.0	NS	0.9	NS	ND	NS	4.8	NS	1.1	**24.0**	5/8 (63)	**38.4**
Beijing 2008	NS	4.1	0.0	ND	0.0	0.4	0.0	0.0	0.9	1.6	ND	0.0	ND	0.0	5.0	7.4	**12.4**	12/15(80)	**19.6**
Chicago 08 1st	NS	ND	NS	1.5	NS	1.2	NS	1.0	NS	5.0	NS	0.7	NS	1.4	NS	ND	**10.5**	6/8 (75)	**14.0**
Chicago 08 2nd	NS	4.4	NS	4.9	NS	4.7	NS	2.1	NS	18.6	NS	2.9	NS	4.2	NS	3.2	**45.0**	7/8 (88)	**48.8**
Chicago 2009	NS	0.5	NS	1.0	NS	2.3	NS	1.9	NS	ND	NS	1.0	NS	3.7	NS	0.0	**10.5**	7/8 (88)	**12.0**
London 2006	NS	ND	NS	7.0	NS	7.0	NS	5.0	NS	6.0	NS	ND	NS	11.0	NS	2.0	**38.0**	6/8 (75)	**50.6**
London 2007	3.0	3.0	5.0	0.0	2.0	3.0	2.0	2.0	5.0	1.0	1.0	2.0	2.0	0.0	0.0	0.0	**31.0**	17/17(100)	**31.0**
New York 2008	NS	1.5	NS	0.0	NS	0.0	NS	0.0	NS	ND	NS	0.0	NS	0.0	NS	0.0	**1.5**	8/9 (89)	**1.6**
Rotterdam 2010	NS	NS	NS	1.9	NS	NS	NS	ND	NS	NS	NS	0.8	NS	NS	NS	0.0	**2.7**	3/4 (75)	**3.6**
Marathon	NS	**5km**	NS	**10km**	NS	**15km**	NS	**20km**	NS	**25km**	**28km**	**32km**	**33.5km**	**36km**	**38km**	**41km**	**Total**	**Stations**	**Extrap**
Fukuoka 2009	NS	4.6	NS	5.0	NS	2.7	NS	4.1	NS	4.2	0.0	ND	ND	ND	ND	0.0	**20.5**	7 /11(64)	**32.3**

* *Note:* NS, no drinking station; ND, no data collected.

To supplement this analysis physical data were collected from the Dubai marathon in 2009. Accordingly, BM of the race winner was recorded to the nearest 0.1 kg prior to the start (just before the start of the race) as well as at the end of the Dubai 2009 marathon and before he consumed any post-race food or drink. Additionally, 5 elite male middle- and long-distance runners (BM: 54.9 ± 3.8 kg; including 2 of the 10 marathon runners investigated while the other 3 specialised in the 5 and 10 km events and included 2 of the world's fastest 5 and 10 km athletes; e.g., world record holder for the 5 and 10 km events) underwent acclimation training in preparation for the 2008 Beijing Olympics. This unique opportunity allowed the assessment of BM losses and consequently, the estimation of sweat rate/losses following 30-60 min of treadmill running at variables speeds ranging from 10-20 $km \cdot h^{-1}$ at 1% gradient to simulate outdoor running (Jones & Doust, 1996). The data collected were used to estimate total BM loss during a hypothetical 02:06:31 (h:min:s) marathon; the average marathon time of the current 10 elite marathoners. These measurements were collected under hotter and more humid conditions (i.e., 35 °C and 70% relative humidity) than typically experienced in major marathon races in which very fast performances are achieved. The hot, humid and wind-still conditions of these experiments limit the extent this data can be extrapolated to out-of-doors running where facing air velocities are seldom lower than the athlete's rate of forward progression (Gonzalez, et al., 2009). Nevertheless, power output (running speeds ranged from 10-20 $km \cdot h^{-1}$ but the majority of time was spent running at 10-12 $km \cdot h^{-1}$; ~14 $W \cdot kg^{-1}$) during these acclimation sessions conducted to mimic ambient conditions expected at the 2008 Beijing Olympics were estimated to be no greater than 50% of the power output at 20 $km \cdot h^{-1}$ (average speed of the marathons assessed; ~30 $W \cdot kg^{-1}$) (Fukunaga & Matsuo, 1980). The higher sweat rates elicited during the acclimation sessions were estimated to be twice as high as those expected during ambient conditions typical of fast marathon racing (e.g., at 16 ^{o}C and 54% relative humidity) (Gonzalez, et al., 2009). Consequently, the hot, humid and wind-still conditions of the acclimation experiments would have elicited maximal or near maximal sweat rates (Nielsen, 1996) and therefore be in line with expected sweat rates during intense marathon racing. Notably, the sweat rate of the winner of the Dubai marathon in 2009 was estimated at 3.6 $L \cdot h^{-1}$ and matched reasonably well with the same athlete's estimated sweat rate during the acclimation session (i.e., 3.2 $L \cdot h^{-1}$).

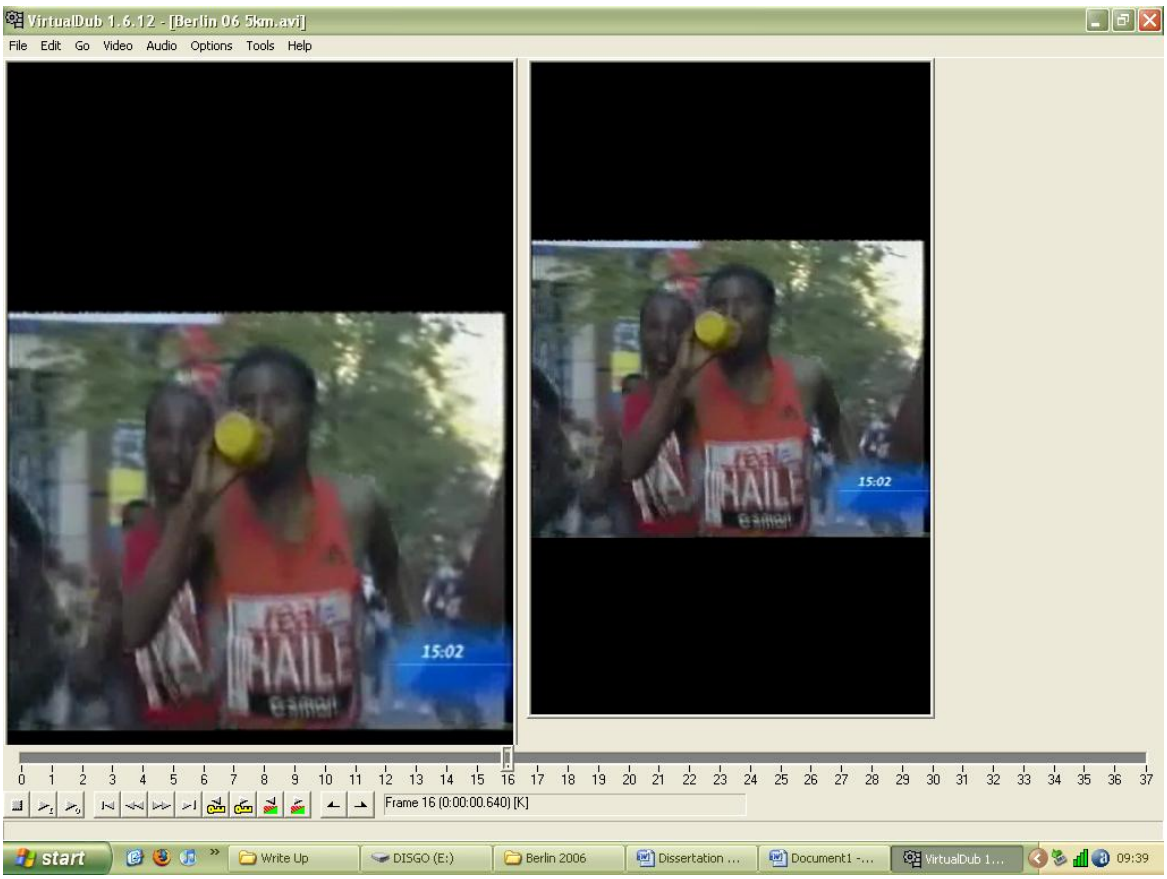


Figure 4.1 Illustration of a snapshot during data collection.

4.2.2 Data Analysis

Data were expressed as mean ± SD, as appropriate following a test for the normality of distribution. Linear regression analysis was performed to investigate the possible association between total drink duration, fluid intake (rate and total), running speed and ambient temperature. Statistical significance was set at $P < 0.05$. Test-retest reliability of the observed total drink duration for both the marathons and the simulation experiment were estimated by the mean of the Spearman-Brown coefficient. Values ≥ 0.7 were considered acceptable reliability. The test-retest procedure was used to assess stability over time after a three-week interval. All statistical analysis was completed using the software package SPSS, version 15.0 (SPSS, Inc., Chicago, IL, USA).

4.3 Results

During the 13 marathon races that we analyzed, the athletes' running speeds ranged from 19.4 $km \cdot h^{-1}$ (Athens 2008) to 20.4 $km \cdot h^{-1}$ (Berlin 2008) with average race time (h:min:s) of 02:06:31. Ambient conditions ranged from 0 ± 1 °C (Chicago 2009) to 30 °C (Athens 2004) and from 29.5 ± 12.5% (Berlin 2006) to 88.5 ± 11.5% relative humidity (Berlin 2008). Invariably, there was no footage available for some drinking stations. Therefore, the obtained data have been extrapolated to provide information for the entire races including the stations for which data were not available. In summary, 72% of all drinking stations were observed on the video footage and data from the analysed footage was extrapolated to 100% (Table 4.2). The mean extrapolated duration of fluid consumption was 25.5 ± 15.0 s and ranged from 1.6 s (New York 2008) to 50.7 s (London 2006) (Table 4.2). Applying a flow rate of 45.2 $mL \cdot s^{-1}$ to these data (Table 4.2), resulted in estimated fluid intakes for the athletes of 1151.3 ± 716.0 mL. These extrapolated data equated to the mean fluid consumption rate (0.55 ± 0.34 $L \cdot h^{-1}$) which falls within the ACSM guidelines (0.4 - 0.8 $L \cdot h^{-1}$; Figure 4.2). Test-retest scores of the observed total drink duration for both the marathon drinking time and the simulation experiment were highly correlated ($r > 0.9$). No significant correlation was found between running speeds and the total time of consuming fluid during the different marathons. The composition of each athlete's drink was unknown except that ingested by the winner of the 2009 Dubai marathon. The composition of the drink ingested during the race consisted of 160 g CHO and 1.2 g Sodium/Natrium per Liter of solution.

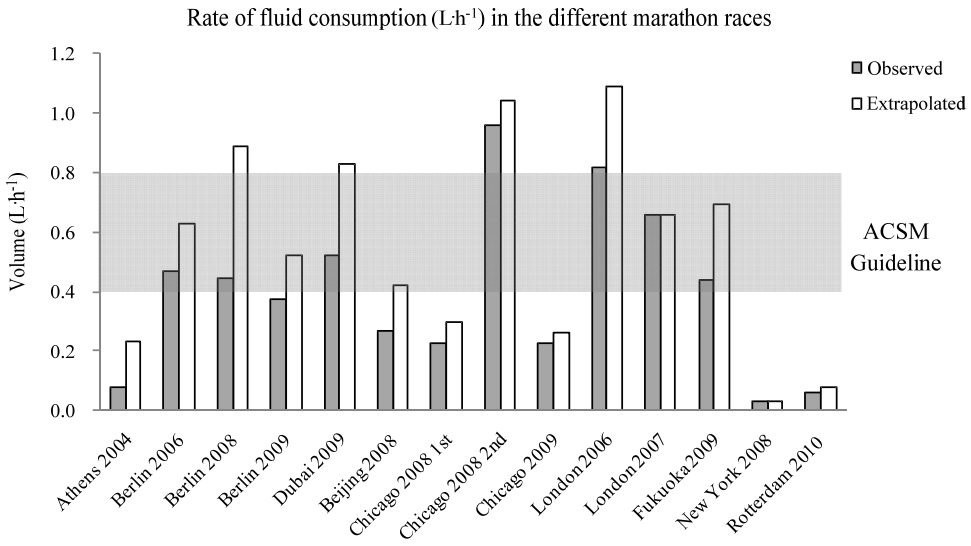


Figure 4.2 The observed and extrapolated rates of fluid consumption ($L \cdot h^{-1}$) of the winning runners in the different marathon races. The grey area represents the ACSM guideline fluid intake rates of 0.4 - 0.8 $L \cdot h^{-1}$.

Measured data collected from the 2009 Dubai marathon (16.0 °C and 53.5% relative humidity) showed that the race winner finished the marathon with a reduction in BM of 5.7 kg (58.2 - 52.5 kg) equivalent to a 9.8% BM reduction. Given the athlete ingested 1.7 L during the race, his estimated sweat rate was approximately 3.6 $L \cdot h^{-1}$. That is similar to his sweat rate (3.2 $L \cdot h^{-1}$) measured during the laboratory experiments (Table 4.3), which were conducted 4 months before the 2009 Dubai marathon. Finally, based on the observed rates of fluid ingestion the estimated BM loss of the 5 elite marathoners, whose sweat rates were measured during the laboratory simulation as 2.3 ± 0.7 $L \cdot h^{-1}$ (range: 1.6 - 3.2 $L \cdot h^{-1}$, Table 4.3), was found to be 8.8 ± 2.1% (range: 6.6 - 11.7%; Table 4.3) during a marathon.

Table 4.3 Measured body mass changes during ~30 min exercise bout in the heat; estimated changes in 5 athletes and observed changes in 1 athlete during 42 km marathon races.

	Measurements during ~ 30 min exercise bout in the heat					Values estimated for a 42km marathon		Observed values during 42km marathon race	
Subject	**Pre BM**	**Post BM**	**Delta BM**	**Exercise Time (min)**	**BML (Kg/h)**	**BML (Kg)**	**BML (%)**	**BML (Kg)**	**BML (%)**
01	59.0	57.6	1.4	30.3	2.7	5.8	9.9		
02	52.6	51.7	0.9	35.4	1.6	3.5	6.6		
02[b]	52.7	51.7	1.1	39.4	1.6	3.5	6.6		
03*	57.0	53.9	3.2	60.0	3.2	6.7	11.7	5.7	9.8
04	57.8	56.2	1.5	36.6	3.0	6.3	10.9		
04[b]	57.1	55.0	2.1	61.1	2.2	4.7	8.2		
05**	48.4	46.8	1.6	56.7	1.7	3.7	7.5		
MEAN	**54.9**	**53.3**	**1.7**	**45.6**	**2.3**	**4.9**	**8.8**	**5.7**	**9.8**
SD	**3.8**	**3.6**	**0.8**	**13.1**	**0.7**	**1.4**	**2.1**		

*Note: SD, standard deviation; BM, body mass; BML, body mass loss; [*] same athlete as subject 02 in Table 4.1; ** same athlete as subject 09 in Table 4.1; [b] different exercise bout.

4.4 Discussion

This investigation (Chapter 4) provides a unique insight into drinking behaviours of elite runners during major city marathons. The first important finding was the wide range of individual rates of fluid ingestion. The mean extrapolated duration spent ingesting fluid was 25.5 ± 15 s, ranging from 1.6 s (New York 2008) to 50.7 s (London 2006). This variation may reflect the different ambient conditions which ranged from 0 ± 1 °C (Chicago 2009) to 30 °C (Athens 2004) and 29.5 ± 12.5% (Berlin 2006) to 88.5 ± 11.5% relative humidity (Berlin 2008). Notably, the warmer conditions did not always result in greater total drinking durations. For example, during the London 2006 marathon the winning athlete consumed more fluid than during the London 2007 marathon (2290 vs. 1400 mL) even though the 2006 race was run in cooler conditions (10 vs. 18 °C) (Table 4.1). Similarly, the runner in the New York 2008 marathon consumed less fluid (73 mL) than did the winner of the Fukuoka 2009 marathon (1458 mL) even though both races were run at almost identical conditions (6 vs. 7 °C, respectively).

The second important finding was that the estimated fluid intakes of the runners during these races was 1151 ± 716 mL, which equates to a mean fluid intake rate of 0.55 ± 0.34 $L \cdot h^{-1}$, assuming a maximal flow rate of 45.2 ± 13 $mL \cdot s^{-1}$. This estimated fluid intake rate is within the current *ad libitum* fluid intake recommendations of 0.4 – 0.8 $L \cdot h^{-1}$ initially proposed by the International Marathon Medical Directors Association (IMMDA) (Noakes, 2003a) and subsequently adopted by the ACSM (Sawka, et al., 2007) (Figure 4.2). It is recognised, however, that these estimated fluid intake rates (Figure 4.2) represent the maximum rates that might have been ingested since these flow rates are dependent on the pressure applied to the sport drink bottle. Currently, this method is the best indirect measure of the drinking behaviours of successful elite marathon runners.

The athletes who won the London 2007, New York 2008 and Rotterdam 2010 races were the only athletes who did not drink anything between 35 - 42.2 km; a distance that these athletes would have covered in approximately 22 min. The study of Montain and Coyle (Montain & Coyle, 1993) found that the physiological effects of fluid ingestion such as a reduced HR and T_{core} as well as increased plasma volume (PV) may require 40 - 60 min to develop. Consequently, late fluid ingestion in a marathon race may be of limited but albeit unknown psychological value since it may result in retained fluid solely in the gastrointestinal tract. This would simply add unnecessary BM without any clear

performance benefit. Indeed some degree of BM loss may actually be advantageous towards the end of a marathon race as theoretically this BM loss will lower the energy cost of running at the same relative sub-maximal speed (Armstrong, et al., 1985; Coyle, 2004; Fudge, et al., 2006a; Sawka & Montain, 2000). Indeed the study of Armstrong et al. (2006) showed that even though $\dot{V}O_2$ (expressed relative to BM) was not significantly influenced by a loss in BM as high as 5.7%, the fractional utilisation of $\dot{V}O_2$max was 3.2% (86.0 vs. 89.2% $\dot{V}O_2$max) lower compared to the euhydrated state (Armstrong et al., 2006). Whilst this difference was not statistically significant, it may be of significance to an elite runner completing the last part of a marathon race, as this difference may influence performance (Hopkins, Hawley, & Burke, 1999). Indeed, it has been shown that athletes who lose the most BM during marathon and ultramarathon races and ironman triathlons are usually the most successful (Cheuvront & Haymes, 2001a; Muir, et al., 1970; Pugh, et al., 1967; Sharwood, et al., 2004; Wharam, et al., 2006; Wyndham & Strydom, 1969; Zouhal, et al., 2010; Zouhal, et al., 2009). Furthermore, there is no evidence that fluid intake during exercise increases sweat rates, stroke volume or reduces overall cardiovascular strain. Nor does fluid intake during exercise alter skin and muscle blood flow, increase oxygen and substrate delivery to the working muscle (Fudge, et al., 2006a).

The data of Chapter 4 suggest that elite marathon runners ingest fluid within a wide range (30 - 1100 $mL \cdot h^{-1}$) during marathon competition. Nevertheless, physical data collected during the Dubai 2009 marathon showed that such rates of fluid ingestion are not sufficient to prevent significant BM losses. Thus, the BM loss of the winner was 9.8% (5.7 kg), even though his rates of fluid intake exceeded the upper limit of the current guidelines (Figure 4.2). This estimated sweat rate was approximately 3.6 $L \cdot h^{-1}$, closely matching his sweat rate measured during a laboratory trial in the heat (3.2 $L \cdot h^{-1}$; Table 4.3). Most probably the laboratory experiments (i.e., acclimation training) which were conducted under fairly extreme ambient conditions (35 ^{o}C, 70% relative humidity and wind-still conditions) and marathon racing at high running speeds (i.e., 20.1 $km \cdot h^{-1}$) would have elicited near maximal sweat rates (Nielsen, 1996). These sweat rates are approximately two times higher in comparison to the highest values of 1.8 $L \cdot h^{-1}$ previously reported (Godek, Bartolozzi, & Godek, 2005; Millard-Stafford et al., 1995). As a consequence of these very high sweat rates, recommended fluid ingestion rates did not prevent significant sweat/BM loss and these athletes performed better than their competitors.

Elsewhere it has been argued that drinking *ad libitum* may be the most effective drinking behaviour (Sawka & Noakes, 2007). Drinking *ad libitum* throughout a marathon appears to confer no major disadvantage over drinking to replace all fluid losses or at least up to the maximal amount tolerated (Cheuvront & Haymes, 2001a; Daries, Noakes, & Dennis, 2000; Dugas, Oosthuizen, Tucker, & Noakes, 2009; Kay D & FE, 2003; McConell, Burge, Skinner, & Hargreaves, 1997; Noakes, 2007a; Saunders, et al., 2005), and there is no study to date demonstrating that full fluid replacement is superior to *ad libitum* drinking (Noakes, 2007a). For example, Daries et al. (Daries, et al., 2000) ran 8 endurance runners twice for 2-h with subjects ingesting a CHO electrolyte drink either *ad libitum* or in set volumes that approximated full replacement of sweat loss. These authors found that the higher rates of fluid ingestion did not alter PV and osmolality and did not improve 2-h running performance. Furthermore, Dugas et al. (Dugas, et al., 2009) tested 6 cyclists, each performing 80 km time trials at 5 different experimental conditions with subjects replacing 0%, 33%, 66%, and 100% of the BM lost during an *ad libitum* trial or simply rinsed their mouths. The authors concluded that *ad libitum* drinking may be the optimal fluid replacement strategy, preventing athletes from ingesting too little or too much fluid and achieving the best possible result (Dugas, et al., 2009). More recently, Goulet et al. (Goulet & Dugas, 2010) had concluded using a meta - analysis of relevant studies, that drinking either more or less than *ad libitum* impairs exercise performance. Furthermore, a pre - planned strategy to limit BM loss is not easily calculated as sweat rate is dependent on ambient conditions and race pace which cannot always be predetermined. For example, ambient conditions during the New York City Marathon has previously changed by as much as 17 °C from start to finish in the same race (Cheuvront & Haymes, 2001b) and race pace at the elite level can vary throughout the race.

An important consequence of drinking *ad libitum* is significant BM loss (Cheuvront & Haymes, 2001a; Muir, et al., 1970; Pugh, et al., 1967; Sharwood, et al., 2004; Wharam, et al., 2006; Wyndham & Strydom, 1969; Zouhal, et al., 2010; Zouhal, et al., 2009). The winner of the Dubai 2009 marathon finished the race with a BM loss of 9.8 %. This level of dehydration did not appear to have any adverse effects during this race or in others in which he drank similar volumes (e.g. Berlin 2006 and 2008). The evidence collected in the present study (Chapter 4), is in accordance with the current literature (Muir, et al., 1970; Pugh, et al., 1967). In general, the world's best marathoners and cyclists during elite competition seem to consume inadequate amounts of fluid in order to prevent more than 2-3% dehydration (Noakes, 2007a). In one of the hottest marathons in recent history (Athens

2004), the winner Mizuki Noguchi appeared to drink relatively small amounts; a total of only approximately 30 s drinking during the race (Van Rooyen, et al., 2010). Hence, it is almost certain that the Olympian had lost a significant volume of body water by the end of the marathon, yet she won the race.

4.5 Conclusions

This study aimed to describe the drinking behaviours of successful elite marathon runners and in the process assess the efficacy of current fluid intake recommendations (albeit indirectly), which propose drinking *ad libitum* 0.4 - 0.8 $L \cdot h^{-1}$ during exercise for elite marathon runners. Although the estimated fluid intake rates of the winners during the 13 races (Table 4.2) are broadly within current recommendations (Sawka, et al., 2007), these elite runners do not seem to maintain their BM during successful marathon racing within current recommended ranges of 2-3%. On the other hand, this apparently widely adopted *ad libitum* strategy during marathon racing seems to produce optimal/winning performances. This evidence and the finding that the winner of the 2009 Dubai marathon finished a competitive race with a BM loss of 9.8% dehydrated, would suggest that there exists a tolerable range for dehydration that may not negatively impact on running performance, but may even confer an advantage by preventing a significant increase in BM due to the "over - consumption" of large volumes of fluid. This finding is in accordance with a recent meta-analysis which suggests that *ad libitum* drinking produces optimum performance (Goulet & Dugas, 2010).

5. The effects of hyper-hydration on running economy in well trained endurance runners

5.1 Introduction

RE, which is defined as the sub-maximal $\dot{V}O_2$ at a given running velocity, is an important physiological parameter as superior RE is essential for successful endurance running performance (Bassett & Howley, 2000; Saunders, et al., 2004). In general, runners with good RE use less oxygen than runners with poor RE at the same absolute exercise intensity. RE appears to be influenced by many physiological factors (Saunders, et al., 2004) including hydration status. Coyle (2003) proposed that a 4 to 8% BM deficit due to dehydration (i.e., the process of reducing body water) may lower the oxygen cost of movement (Coyle, 2004), given that athletes who lose the most BM during a race are usually the most successful (Zouhal, et al., 2010). Nevertheless, this theoretical paradigm contradicts the prevailing view of a body water deficit in excess of 2-3% BM constituting the level of dehydration that can adversely affect performance (Sawka, et al., 2007).

During exercise, skeletal muscle produces a significant amount of heat. When this metabolic heat production exceeds total heat loss, T_{core} rises. Consequently, endurance exercise performance in hot and dry environments can be limited by the increase in T_{core} (Nielsen et al., 1993). An increase in T_{core} during exercise can be attenuated via the secretion and evaporation of sweat through the skin with inevitable body water loss. This decrease in body water is hypothesized to decrease PV and consequently reduce the sweating response and therefore thermoregulation capacity, increase HR and reduce skin blood flow (Ekelund, 1967). Improved maintenance of PV is the overriding rationale for fluid ingestion during exercise by those supportive of the "cardiovascular model of dehydration" (Sawka, et al., 2007). However, proposed guidelines (Sawka, et al., 2007) are not always practical (e.g., difficulties providing adequate drinks during a race, athletes difficulties in drinking while running) and athletes typically refrain from consuming recommended amounts of fluids. Other means to expand PV can be by infusion of isotonic saline (Fortney, Vroman, Beckett, Permutt, & LaFrance, 1988) with somewhat conflicting success (Fortney, et al., 1988; Grant, Green, Phillips, & Sutton, 1997). More recent approaches aimed at expanding body water compartments using hydrating agents such as Cr and Gly have successfully attenuated the rise in T_{core} and HR during exercise in heat (Magal, et al., 2003; Riedesel, et al., 1987).

Cr has been shown to have hydrating effects (Kern, et al., 2001; Kilduff, et al., 2004), although the exact process has yet to be established. Ingestion of 20 $g \cdot day^{-1}$ of Cr dissolved in 500 mL of water for 7 days have proved successful in attenuating the rise in HR and T_{core} during exercise in the heat (Kilduff, et al., 2004). These effects have been attributed to an increase in ICW, resulting in an increased specific heat capacity of the body (Kern, et al., 2001; Kilduff, et al., 2004). Moreover, whole body Cr retention is 60% higher when consumed with CHO compared to when Cr was consumed alone (Green, Hultman, Macdonald, Sewell, & Greenhaff, 1996). Although the mechanism by which CHO enhances Cr uptake is not completely understood, consumption of 100 g per 5 g of Cr has been recommended for the effective improvement of Cr uptake (Steenge, Simpson, & Greenhaff, 2000). Like Cr, Gly has been found to be an effective agent in expanding the water compartments within the human body (Murray, et al., 1991; Riedesel, et al., 1987). Gly, seems to expand the ICW as well as the ECW (Nelson & Robergs, 2007). In general, doses of 1.0–1.5 g $Gly \cdot kg^{-1}$ BM dissolved in 1.4 - 2.0 L of fluid 2.5 - 4 h before exercise (van Rosendal, et al., 2010) increase TBW compartments and reduce thermal and cardiovascular strain during exercise in the heat.

Supplementation with combined hydrating agents such as Gly or Cr has consistently produced modest fluid retention of 400 - 800 mL (Kern, et al., 2001; Magal, et al., 2003; Riedesel, et al., 1987). Easton et al. (2007) were the first to add Gly to a Cr containing solution and demonstrate that a combination of the two hyperhydrating agents has an additive effect, as the addition of Gly to Cr significantly increased TBW more than Cr alone. Although the combination of the aforementioned hyperhydrating agents results in an increase in TBW and a reduction in certain cardiovascular and thermoregulatory responses (Easton, et al., 2007), the BM increase due to enhanced hydration status could potentially reduce RE. The reduction of the energy cost of movement at a sub-maximal velocity by way of reducing BM to improve running performance is well known (Fudge, et al., 2006b). For instance, it is noted that some marathon runners perform well despite dehydration of 4 - 8% BM (Costill, 1972). Coyle (2004) proposed that this may occur because fluid loss (i.e., reduced BM) lowers the oxygen cost of movement. On the other hand, the acute influences of hyper-hydration on RE has not been investigation to date. Hence, the aim of the study presented in Chapter 5 was to investigate the effects of hyper-hydration induced by a combined Cr and Gly supplementation on thermoregulatory and cardiovascular responses and RE during 30 min of running at a running speed corresponding to 60% $\dot{V}O_2max$ in cool (10 °C with a relative humidity of 70%) and hot

conditions (35 °C with a relative humidity of 70%) in well-trained male athletes. In cool ambient conditions were intended to minimize heat stress during exercise thus enabling a focus on the effects of the altered BM induced by hyper-hydration on RE at 60% $\dot{V}O_2$max. However, effects of hyper-hydration on thermoregulatory and cardiovascular responses are also expected during exercise in hot and humid conditions; conditions typical of major sporting events (e.g., Olympic Summer Games). As such, it was hypothesized that an increase in BM and TBW induced by hydrating agents such as Gly or Cr would improve thermoregulatory and cardiovascular responses in line with previous findings but potentially negatively influence RE during running in the heat.

5.2 Methods

5.2.1 Subjects

Fifteen well-trained male runners gave their written informed consent to take part in the present observational, non-blinded, non-randomised study (Chapter 5). The current investigation was approved by the University of Glasgow Ethics Committee and was performed in the Glasgow University according to the code of ethics of the World Medical Association (Declaration of Helsinki). One subject withdrew from the study before the final trial because of gastrointestinal distress during supplementation. Subjects were questioned as to their supplementation and training practices in order to ascertain that they had not supplemented with Cr for at least 8 weeks prior to commencing the study. Subjects were in good health at the time of testing, ran on a daily basis and participated regularly in competitive races. Athletes were also requested to maintain their typical weekly training regime during the course of the study.

5.2.2 Experimental Procedures

All subjects completed a $\dot{V}O_2$max test during an initial continuous incremental test at standard room temperature (20 - 21 °C) and relative humidity (30 - 40%) on a motorized treadmill (PPS Med, Woodway, Germany) at 1% grade. After a warm-up period (depending on the runner), the subjects started running at 8 $km \cdot h^{-1}$ for 3 min in order to reach a steady state. In the next exercise bout the treadmill speed was set to 10 $km \cdot h^{-1}$ for 3 min and this procedure was repeated with 2 $km \cdot h^{-1}$ increments in running speed until volitional exhaustion of the subject. During the test expired gas samples (30 s collection time at the end of each bout) were taken using Douglas bag collection technique as is considered the gold standard method (Douglas, 1911) and analyzed for O_2% and CO_2%

(Servopro 4100 Gas Purity Analyzer, Servomex, UK) as well as analyzed for volume using a dry gas meter (Harvard, Kent, UK) and temperature of expired gases. Barometric pressure was measured using a standard mercury barometer. Additionally, a HR monitor (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) was attached prior to each test and HR was recorded at the end of each bout. The $\dot{V}O_2$max measurement was used for calculating the intensity (60% of $\dot{V}O_2$max) that subjects would perform during the actual tests. Running speed at 60% of $\dot{V}O_2$max (exercise intensity) was calculated using the linear relation between treadmill speed and $\dot{V}O_2$.

Prior to the actual experimental trials, familiarization trials were completed until the variability of $\dot{V}O_2$ of two consecutive trials was within 5% difference. No subject had to complete a third familiarization trial to achieve less than 5% variability, an observation which is in line with our previous experience of trained athletes (Easton, et al., 2007). At least three days after this familiarization period, subjects reported to the laboratory for the first experimental trial (i.e., a pre-supplementation trial). After this baseline test, all subjects commenced the hyper-hydration treatment comprising Cr, Gly and glucose. For this, subjects consumed a solution of 11.4 g of Cr·H_2O (equivalent to 10 g Cr), (Reflex Creapure Creatine, Reflex Nutrition LTD, UK), 1 g·kg^{-1} of BM Gly (Glycerin BP/Value Health Glycerin BP, Boots Company plc) and 75 g of Glucose polymer (SiS GO electrolyte), mixed in hot water (approximately 50 °C) and made up in 1 L of cold water twice daily. This supplementation regimen was followed for 6 days. This protocol has been shown to increase resting muscle-phosphocreatine levels within 5 days (Harris, Soderlund, & Hultman, 1992). On the day of the post-supplementation test (i.e., day 7th) subjects began consuming the final supplement 5 h before the exercise-performance trial (with instructions to complete ingestion within 1 h). Hypertonic solutions such as the Cr, Gly, Glucose combination (~1556 mOsm·kg^{-1}) cause an initial net secretion of water into the intestinal lumen (Gisolfi, Summers, Schedl, Bleiler, & Oppliger, 1990), resulting in an effective loss of body water, albeit temporary. Unpublished work from our laboratory has indicated that ingesting Cr/Gly 5 h prior to commencement of exercise results in a larger volume of fluid absorbed compared to when the solution is consumed 3 h prior to the exercise test. In order to prevent degradation of Cr to creatinine, each supplement was prepared fresh each time before consumption. The subject was also given a temperature pill (HQ Inc., USA*)* about 8-12 h prior to each test allowing T_{core} to be measured (Easton, Fudge, & Pitsiladis, 2007). On each of the experimental test days, subjects ingested 500 mL of water 1 h before exercise in an attempt to ensure euhydration before all exercise

trials (Convertino, et al., 1996). Subjects otherwise followed their normal diet and recorded all food and drink consumed during the supplementation period as well as the preceding week using a food diary. The diet was analyzed for EI and macronutrient content using computerized food composition tables (Holland, 1991) (Food Meter U.K., Medimatica s.r.l., Benedetto, Italy). Subjects were asked to minimize caffeine intake to 1 cup of tea or coffee per day to lessen any possible confounding effects of caffeine on Cr (Vandenberghe et al., 1996).

The subject reported to the lab after a 3 h fast and having refrained from alcohol, caffeine, and strenuous exercise at least 24 h prior to the experimental trial. Firstly, a urine sample was collected from the subject prior to taking the pre-test nude BM (Tanita Corporation of America, Inc.). Body water compartments were estimated using a multi frequency bioimpedance analyzer while the subject lay comfortably in a supine position for 5 min on a nonconductive surface with their arms and legs slightly abducted (Quadscan 4000, Bodystat Ltd., Isle of Man). This method allows TBW and ECW to be estimated. From these measurements ICW can also be deduced. Bioimpedance has been shown to produce valid and reliable TBW estimations in the euhydrated state (O'Brien, Young, & Sawka, 2002) which has been also verified in the present lab (unpublished measurements; highly correlated with deuterium oxide measurements). To date, several studies have successfully used this technique in order to estimate hyper-hydration induced changes in TBW (Kern, et al., 2001; Kilduff, et al., 2004). Changes in BM from pre- to post-supplementation were used to supplement the indirect measurement of the fluid volume retained. Following TBW determination, the subject lay in a supine position for 5 min further and a 7 mL blood sample was taken from a 21G cannula which was introduced into a superficial vein of the anticubital fossa of the right arm. The venous cannula was kept patent by flushing it with 7 mL of isotonic saline solution between samples. Prior to entering the environmental chamber a HR monitor (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) was attached to the subject. Then, the subject was transferred to the climatic chamber (ambient temperature 10.0 ± 1.0 °C with a relative humidity of 68.5 ± 3.6%). Subjects were then instructed to begin running to their predetermined 60% $\dot{V}O_2max$ for 30 min at 1% inclination of the treadmill. HR and T_{core} were recorded every 5 min throughout the 30-min exercise period. 1 min gas measurements were collected at 5 min intervals of exercise for the purpose of $\dot{V}O_2$, carbon-dioxide production ($\dot{V}CO_2$), temperature and expired gas volume determination. RPE and thermal comfort (TC) were recorded every 5 min of the exercise using the Borg category scale (Borg, 1982) for RPE and a modified scale (from -

10 to + 10). Following the first exercise bout, the subject was removed from the chamber and nude BM was measured immediately. The difference in BM before and after exercise was calculated and subsequently used to estimate sweat rate and sweat loss. Subsequent to BM determination, the subject lay in a supine position for 10 min and a final blood sample was retrieved. The fluid loss was then replaced by giving the subject the equivalent amount of water to that calculated between pre- and post-exercise. Subjects were then instructed to re-enter the climatic chamber and complete a second bout of run at the same speed (60% $\dot{V}O_2max$), at 35.1 ± 0.1 °C and 69.4 ± 4.0% relative humidity. The protocol for data collection was identical to the one used in the first bout of exercise. Once the second bout was completed, subjects' nude BM and a final blood sample were taken as described above. The analytical procedure is shown in Figure 5.1. Illustrations also from the lab work are presented in Figure 5.2.

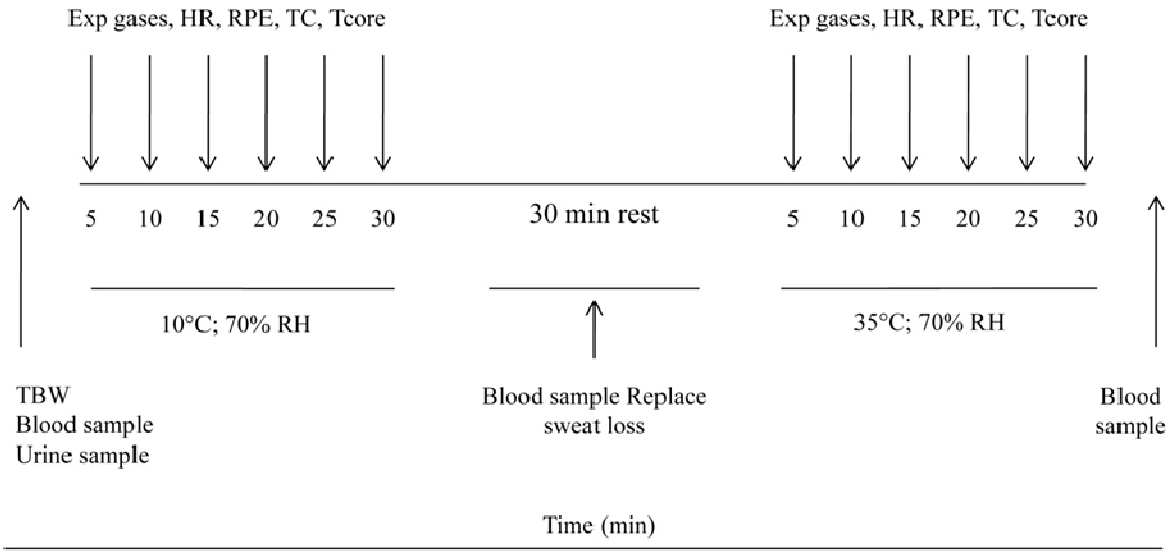


Figure 5.1 Schematic representation of the experimental protocol.

5.2.3 Blood Analysis

Blood was drawn into dry syringes and 4 mL dispensed into a tube containing K_3EDTA and the remaining 3 mL dispensed into plain tubes. Duplicate aliquots (100 µL) of whole blood from the K_3EDTA tube were rapidly deproteinised in 1000 µL of ice-cold 0.3-mmol/L perchloric acid, centrifuged (8 min, 14000 rpm, Hettich Microcentrifuge, Germany), and frozen for later analysis of lactate using a standard enzymatic method (Maughan, 1982) involving fluorimetric detection (Spectramax M2 Microplate Reader, Molecular Devices, Inc., US). The blood in tubes without anticoagulant was allowed to coagulate and then centrifuged; the serum collected was used to measure osmolality by

freezing-point depression (Micro-osmometer 3300, Vitech Scientific, West Sussex, UK). The blood from the K_3EDTA tubes was also analyzed for haemoglobin (cyanmethemoglobin method) and packed-cell volume (PCV; conventional micro-haematocrite method). All blood analyses were carried out in duplicate, with the exception of PCV, which was carried out in triplicate. PV changes were calculated from changes in haemoglobin and PCV relative to initial baseline values (Dill & Costill, 1974).

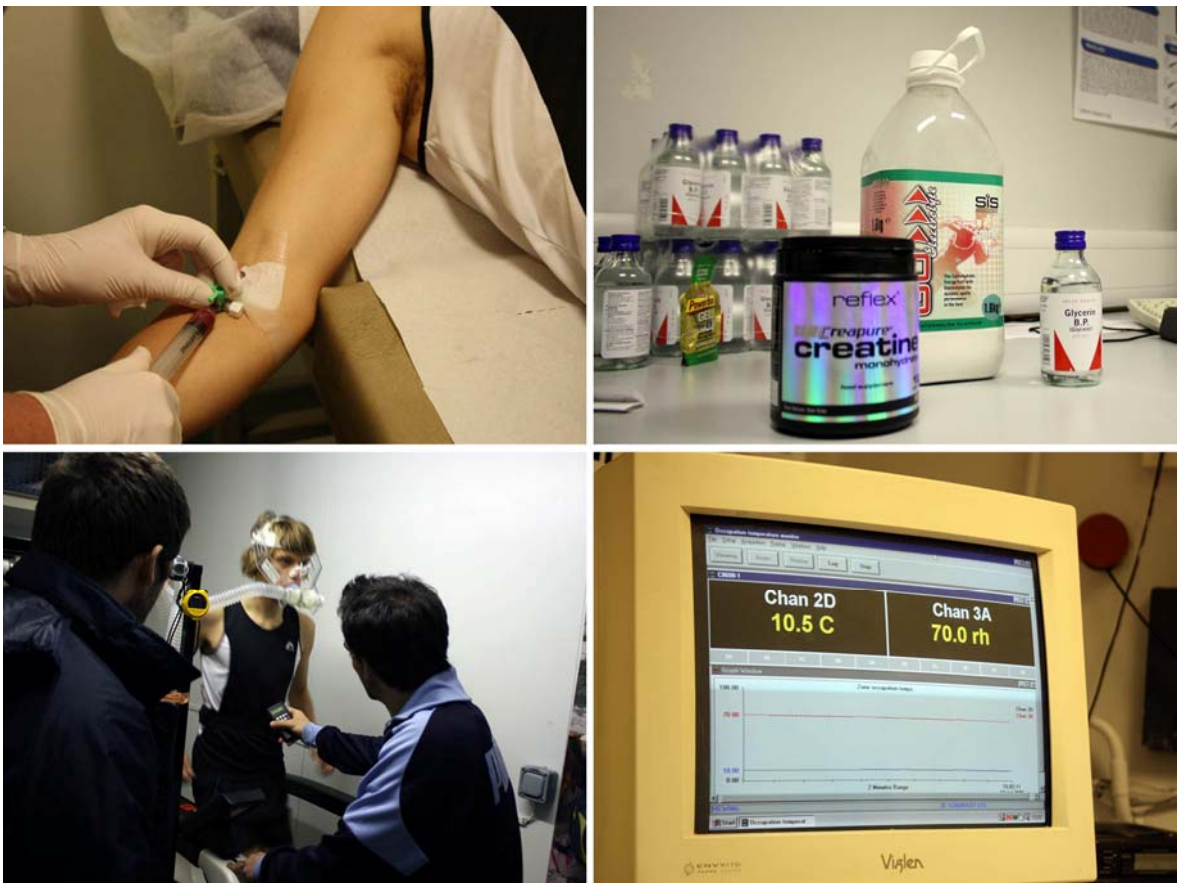


Figure 5.2 Illustrations of the lab work data collection.

5.2.4 Data Analysis

All data are expressed as the mean ± SD. All experimental variables ($\dot{V}O_2$, $\dot{V}CO_2$, Respiratory exchange ratio (RER), RPE, TC, HR, T_{core}) were tested for normality of distribution and compared between the two treatments using two-way ANOVA (i.e., pre- vs. post-supplementation). Students paired t-tests were carried out to test difference between each time pre- to post-supplementation when difference was detected using ANOVA. Statistical significance was set at $P < 0.05$ and in cases where significant differences were detected between time points pre- to post-supplementation, *P*-value was

corrected using the Sidak adjustment. Responses at 10 and 35 ^{o}C were analysed separately. Student paired t-tests were also used to examine the difference between pre- to post-supplementation for the rest of the comparisons. All statistical analysis was completed using the statistical package SPSS, version 15.0 (Statistica 8.0, Statsoft Inc., Tulsa, USA).

The intra-assay C.V. was calculated from the SD of the difference between double or triple (PCV) measurements of the sample expressed as a percentage of the total mean sample (Table 5.1).

Table 5.1 Coefficient of variation of blood and serum assays.

Assay	Method	*N*	C.V.
Blood lactate	Maughan 82	14	4.7
Serum osmolality	Freezing point depression	14	0.2
Blood Hb	Calorimetric method	14	0.5
PCV	Microhaematocrit method	14	0.5

5.3 Results

5.3.1 Subject characteristics

The 15 male subjects were trained distance runners with $\dot{V}O_2max$ being 63.5 ± 5.2 mL·kg^{-1}·min^{-1}, age, 24 ± 5 yr; height, 180 ± 7 cm; BM, 69.5 ± 5.0 kg (values are presented as the mean ± SD).

5.3.2 Body Mass and Water Compartments

Supplementation induced significant increase in BM, TBW, ICW and ECW ($P < 0.01$; Figure 5.3). During supplementation period as well as the preceding week averaged daily EI (Pre: 12.8 ± 2.1 MJ·day^{-1}; Post: 11,5 ± 2.4 MJ·day^{-1}) and averaged proportion of energy obtained from CHO (Pre: 55 ± 5%; Post: 49 ± 11%), fat (Pre: 33 ± 5% Post: 36 ± 6%), and protein (Pre: 13 ± 1%; Post: 14 ± 3%) were not significant different.

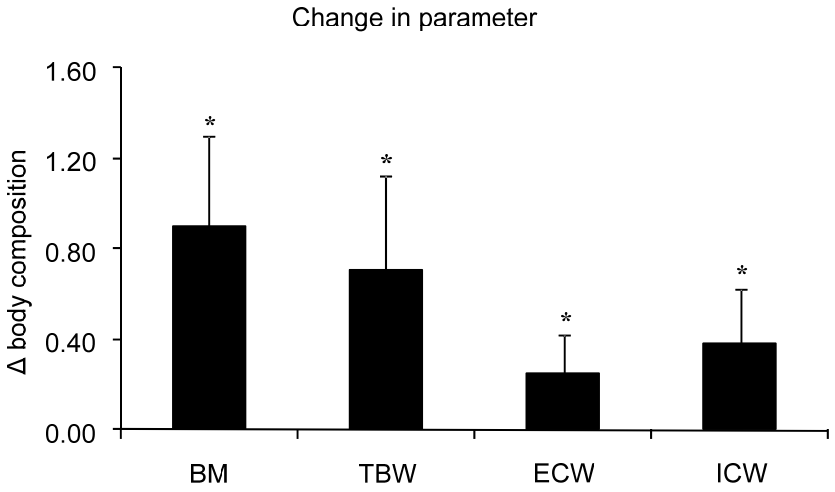


Figure 5.3 Changes in body mass (BM), total body water (TBW), extracellular water (ECW) and intracellular water (ICW) induced by supplementation. Data presented as mean ± SD. *Significant difference between pre- and post-supplementation.

5.3.3 Cardiopulmonary Variables

Over the duration of running at 10 ^{o}C $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$) and RER remained constant (Table 5.2). Over the duration of running at 35 ^{o}C $\dot{V}O_2$ and $\dot{V}CO_2$ increased significantly ($P < 0.05$, AVOVA, time effect) while the values of RER were constant. No significant differences were detected for $\dot{V}O_2$, $\dot{V}CO_2$, RER between pre- and post-supplementation trials during running at both 10 and 35 ^{o}C (Table 5.2). HR increased significantly over the duration of running at 10 and 35 ^{o}C ($P < 0.05$, for both, ANOVA, time effect). During running at 10 ^{o}C there was no difference in HR between pre- and post-supplementation trials (Figure 5.4). During running at 35 ^{o}C, HR was significantly lower ($P < 0.05$, ANOVA, trial effect) in the post-supplementation trial compared to the pre-supplementation trial.

Table 5.2 Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER) during 30 min of running at 10 and 35 °C conducted before and after supplementation.

			Exercise time (min)					
Variable	**Condition**		**5**	**10**	**15**	**20**	**25**	**30**
$\dot{V}O_2$	10 °C	Pre	37.4±2.4	37.6±2.0	37.7±1.8	38.7±2.2	38.8±2.7	38.9±2.8
($mL \cdot kg^{-1} \cdot min^{-1}$)		Post	36.4±2.8	37.4±1.5	36.9±1.7	37.7±1.8	37.6±2.2	38.4±3.3
	35 °C	Pre[a]	37.2±2.4	39.5±2.4	39.5±2.3	40.3±2.6	40.5±4.4	41.2±3.3
		Post[a]	36.7±2.4	37.9±2.3	37.4±3.2	38.4±2.6	39.1±2.1	38.5±3.1
$\dot{V}CO_2$	10 °C	Pre[a]	32.8±1.7	33.7±2.2	33.9±1.4	34.4±2.0	34.7±2.5	34.4±2.5
($mL \cdot kg^{-1} \cdot min^{-1}$)		Post[a]	33.5±3.1	34.6±1.6	34.0±1.7	35.0±1.9	35.1±2.0	35.0±2.3
	35 °C	Pre	32.3±2.8	34.7±2.3	35.6±2.3	35.3±2.2	35.5±3.2	35.5±3.3
		Post	32.4±2.5	33.9±2.2	34.4±2.4	35.1±2.3	35.1±2.3	34.5±2.6
RER	10 °C	Pre	0.87±0.03	0.89±0.03	0.89±0.03	0.88±0.04	0.89±0.04	0.88±0.03
		Post	0.91±0.05	0.93±0.03	0.92±0.03	0.93±0.03	0.93±0.03	0.92±0.03
	35 °C	Pre	0.87±0.05	0.88±0.03	0.89±0.03	0.88±0.04	0.88±0.05	0.86±0.05
		Post	0.88±0.03	0.89±0.03	0.91±0.03	0.91±0.03	0.90±0.03	0.89±0.03

**Note.* Values are presented as the mean ± SD; [a]Significant difference over time throughout the trial; *P*-value was set at 0.05.

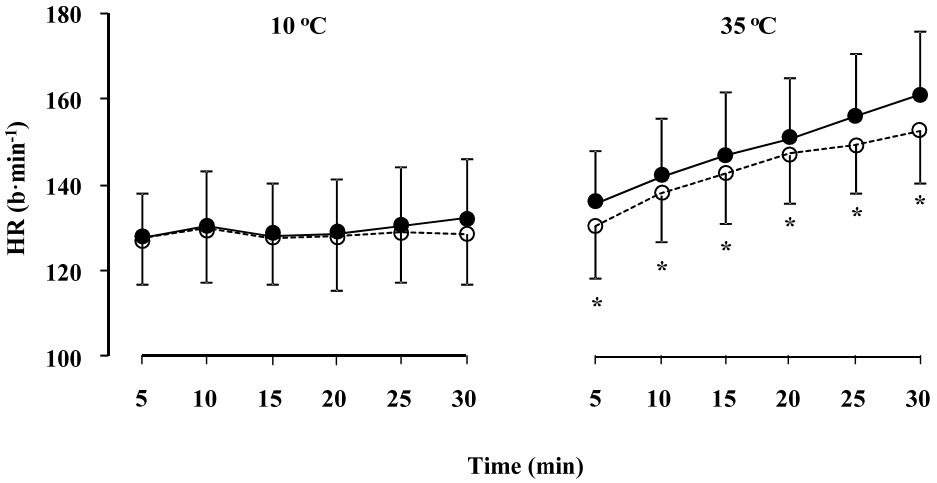


Figure 5.4 Heart rate (HR) during exercise at 10 and 35 °C before (black circles) and after (white circles) supplementation. Data presented as mean ± SD. *Significant difference between pre- and post-supplementation.

5.3.4 Rating of Perceived Exertion (RPE) and Thermal Comfort (TC)

Over the duration of running conducted at both 10 and 35 °C significant ($P < 0.05$, ANOVA, time effect) increases were detected in RPE (Figure 5.5) and TC (Figure 5.6), while no significant differences were found between pre- and post-supplementation trials.

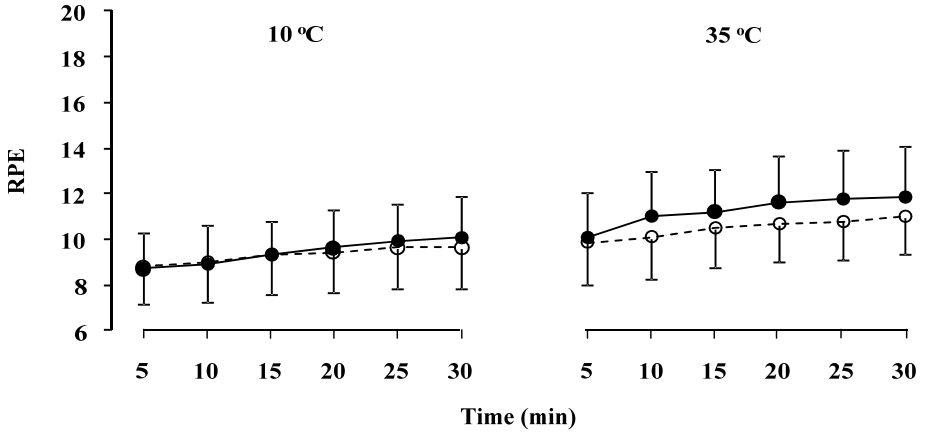


Figure 5.5 Rating of perceived exertion (RPE) during exercise at 10 and 35 °C before (black circles) and after (white circles) supplementation. Data presented as mean ± SD.

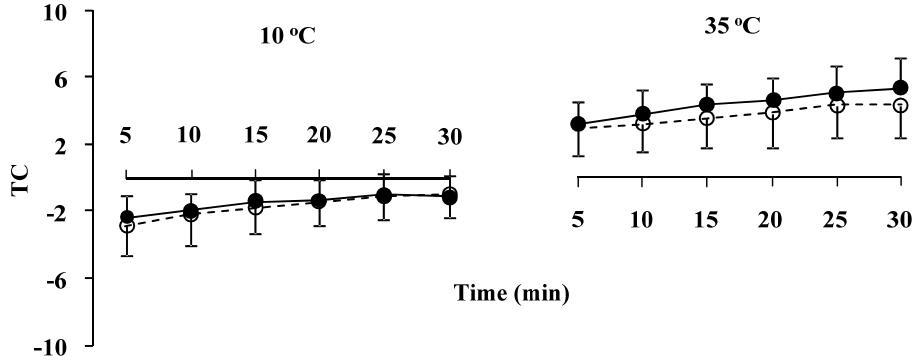


Figure 5.6 Thermal comfort (TC) during exercise at 10 and 35 °C before (black circles) and after (white circles) supplementation. Data presented as mean ± SD.

5.3.5 Core Temperature (T_{core})

Over the duration of running conducted at both 10 and 35 ^{o}C T_{core} increased significantly ($P < 0.05$, for both, ANOVA, time effect) (Figure 5.7). During running at 35 ^{o}C T_{core} was significantly lower ($P < 0.01$, ANOVA, trial effect) in post- than pre- supplementation trial. During running at 10 ^{o}C there was no difference in T_{core} between pre- and post-supplementation trials.

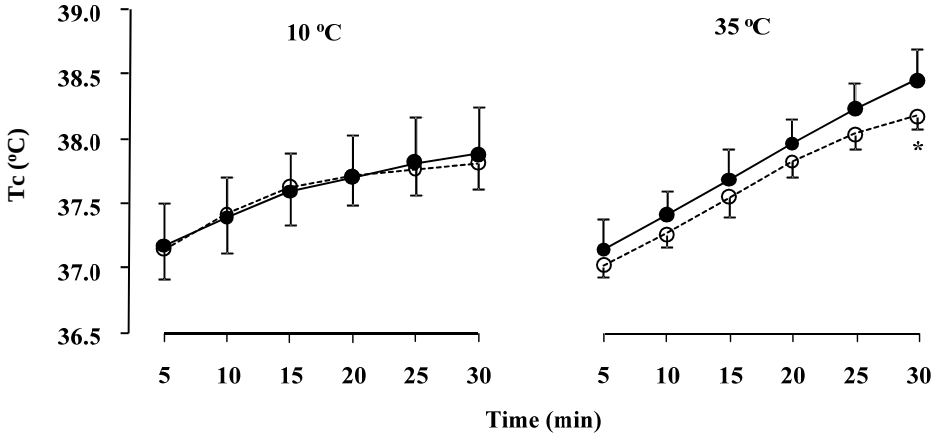


Figure 5.7 Core temperature (T_{core}) during exercise at 10 and 35 ^{o}C before (black circles) and after (white circles) supplementation. Data presented as mean ± SD. *Significant difference between pre- and post-supplementation.

5.3.6 Urine osmolality

No significant changes were found in urine osmolality between the pre- (438 ± 306 mOsm·kg^{-1}) and post-supplementation trials (448 ± 266 mOsm·kg^{-1}).

5.3.7 Total Sweat Loss

During running at 10 °C no significant differences between pre- and post-supplementation trials were observed in sweat loss (Pre: 0.3 ± 0.1 L; Post: 0.3 ± 0.1 L). Similarly, during running at 35 °C no significant differences between pre- and post-supplementation trials were observed in sweat loss (Pre: 0.7 ± 0.2 L; Post: 0.8 ± 0.2 L).

5.3.8 Blood Lactate and Plasma Volume (PV)

During running at both 10 and 35 ^{o}C no significant differences were found between pre- and post-supplementation trials in resting concentration of blood lactate. Furthermore, no significant increase in blood lactate was observed over duration of exercise. Additionally, during running at both 10 and 35 ^{o}C no significant differences were detected between pre- and post-supplementation trials in PV changes.

5.3.9 Osmolality

Resting serum osmolality did not differ between pre- (268 ± 9 mOsm·kg^{-1}) and post-supplementation (271 ± 19 mOsm·kg^{-1}) trials. Additionally, no differences were observed between the post 10 and 35 °C bouts and the resting values or between the treatments.

5.3.10 Side Effects

In general, subjects tolerated the supplementation protocol well, with only 1 report of gastrointestinal distress after supplementation who withdrew from the experimental process before completing the post-supplementation trial. This report is in line with the previous study by Easton et al. (2007), where 1 athlete had to also withdraw from the study due to similar reasons.

5.4 Discussion

The novel finding of this study is that a previously established pre-exercise water loading strategy using a combination of hydrating agents such as Cr and Gly that significantly increased body water compartments and reduced cardiovascular (Figure 5.4) and thermoregulatory (Figure 5.7) responses during running at 35 ^{o}C, had no effect on the oxygen cost of running at 60% of $\dot{V}O_2$max. The magnitude of change in BM following hyper-hydration was similar to that previously reported in our laboratory (Easton, et al., 2007) and by Kern et al. (2001). Somewhat smaller differences in body water compartments were observed in the present study (Chapter 5) compared to the previous investigation by Easton et al. (2007). For example, Easton et al (Easton, et al., 2007) reported an increase of 0.9 L in TBW and 0.5 L in ICW after 7 days of supplementation. In the present study TBW and ICW were elevated by 0.7 and 0.3 L respectively after 7 days of supplementation. These differences could only be attributed to individual

responses (i.e., level of "responders" to Cr supplementation as previously demonstrated) (Kilduff, et al., 2004; Kilduff et al., 2003) as similar protocols were utilised. In the present study, the retained water was dispersed in both the ICW and ECW. Despite the significant increase in BM and body water compartments and consequently improved thermoregulatory responses during exercise, no significant differences in any of the respiratory variables were found between the pre- and post-supplementation exercise trials. Therefore, the finding that a significant increase in BM did not negatively impact on RE of trained runners supports the use of hyper-hydration during endurance running when running in hot and humid conditions although confirmatory results are required during faster running speeds typical of sporting competition (i.e., > 85% $\dot{V}O_2max$).

Temperature and cardiovascular regulation during exercise in the heat do appear to be critically dependent on hydration status (Gonzalez-Alonso, Mora-Rodriguez, Below, & Coyle, 1995; Nadel, Fortney, & Wenger, 1980). In the present study, combined Cr and Gly supplementation induced significant hyper-hydration and substantially attenuated the increase in HR at the end of the 30 min run at 35 ^{o}C (Figure 5.4). This attenuation of HR during exercise was of similar magnitude to that previous reported by Easton et al. (2007). As free water in the form of sweat is primarily lost from plasma and since no differences were found in PV changes pre- and post-supplementation despite the increase in TBW, ICW and ECW, it can be suggested that the increase in other water compartments resulted in water moving towards the plasma due to an osmotic gradient. This in turn leaves the PV unaffected. It should be also noted that in order for blood volume to be maintained in conditions of significant thermal stain and therefore sweating, fluid loss is obtained in varying proportions from ECW as well as ICW body water compartments (Sawka, Montain, & Latzka, 2001). Furthermore, as loss of body water increases during exercise in the heat as a result of sweating, T_{core} also increases (Sawka, et al., 2001). Therefore, increasing body water could potentially result in better maintenance of T_{core} during exercise in the heat. Nose et al. (1988) reported a strong association between the loss of water in sweat and urine and the decrease in ICW after prolonged exercise in the heat (Nose, Mack, Shi, & Nadel, 1988). In the present study (Chapter 5), Cr and Gly induced an increase in ICW and consequently, there was a significant attenuation in the rise of T_{core} during exercise in the heat (Figure 5.7). It is possible that this Cr- and Gly-induced increase in ICW resulted in an increase of the specific heat capacity of the body (Kilduff, et al., 2004).

Published studies to date appear to confirm the reduction of T_{core} during exercise in the heat following Cr supplementation (Easton, et al., 2007; Kern, et al., 2001; Kilduff, et al., 2004). Conversely, when Gly was used alone, ICW was increased without significantly attenuating the rise in T_{core} during the exercise period (Easton, et al., 2007). The effects of Gly ingestion on T_{core} and thermoregulation in general during exercise in the heat is equivocal, with several studies reporting a reduction in T_{core} during exercise (Lyons, et al., 1990) and numerous other studies finding no such effect (Latzka et al., 1998; Murray, et al., 1991). In addition, several studies concluded that PV expansion has no effect on thermoregulatory responses or exercise performance during exercise in the heat (Grant, et al., 1997; Watt, Garnham, Febbraio, & Hargreaves, 2000). These conflicting results and assertions provide strong support that the thermoregulatory benefits exhibited with Gly ingestion in the present study did not arise from any PV expansion but most likely from an increased heat capacity of the body. Nevertheless, it should also be noted that these thermoregulatory benefits were exerted when Gly was co-ingested with Cr.

Despite the significant increase in TBW and consequently improvement in cardiovascular and thermoregulatory responses during exercise, no differences in $\dot{V}O_2$max were observed during running at 60% $\dot{V}O_2$max. Coyle proposed that a reduction in BM induced by dehydration would impact on RE during marathon running by reducing the oxygen cost of running (Coyle, 2004). In contrast, hyper-hydration should theoretically increase the oxygen cost of running and therefore RE. However, no such effect was found in the present study. Furthermore, there was no increase in $\dot{V}O_2$ over time during the trial at 10 °C. The latter finding indicates that the subjects were working steadily at the calculated individual running speed corresponding to 60% of $\dot{V}O_2$max. It should be noted that this relatively low intensity was chosen in order to ensure that the present data would be comparable with previous studies conducted under similar conditions (Kern, et al., 2001). Furthermore, the relatively low intensity was chosen as to secure that all subjects could complete the experiment in the heat while it was high enough to observe possible adaptations in cardiopulmonary or thermoregulatory parameters encountered with supplementation. However, $\dot{V}O_2$max was increased during the trial in the heat. This was an expected effect as when exercising in hot environmental conditions, T_{core} rises accordingly. It has been shown that with an increase in $T_{core,}$ $\dot{V}O_2$ (and therefore RE) also increases (MacDougall, Reddan, Layton, & Dempsey, 1974). Despite this observation, no discernable difference in $\dot{V}O_2$ between pre- and post-supplementation trials was reported. No other changes in any of the respiratory variables could be observed in the pre- and post-

supplementation trials. Similar results have been reported in several other studies using Cr as the hyperhydrating agent (Kilduff, et al., 2004) as well as during constant load exercise in the study by Easton et al. (2007) where hyper-hydration was induced by Cr and Gly (Easton, et al., 2007). The data from Chapter 5 suggest that an increase in BM of approximately 1.4% (average increase in BM in the present study) has no significant effect on $\dot{V}O_2$. Whether such an increase in BM would influence running performance remains to be determined. Furthermore, as HR responses reflect those of $\dot{V}O_2$ (Fudge et al., 2007), the finding that HR during exercise was not significantly different between pre- and post-supplementation trials conducted at 10 °C is further evidence against any detrimental metabolic effect of the added BM induced by hyper-hydration on RE.

5.5 Conclusion

A hyper-hydration strategy that combines Cr and Gly supplementation for 7 days increased BM and TBW and consequently reduced cardiovascular and thermal strain but did not significantly affect the oxygen cost of running at 60% of $\dot{V}O_2$max at 35 ^{o}C in trained runners. The finding that a significant increase in BM did not negatively impact on RE of trained runners, supports the use of effective hyper-hydration strategies during endurance running when conditions so dictate (i.e., running in hot and humid conditions). Further studies are necessary however to confirm these findings during faster running speeds reflective of true performance.

6. General Discussion

The primary objectives of the studies described in the previous chapters were:

1. To compare data extracted from a well-controlled study to previous investigations that did not control for primary confounding factors that could have adversely affected the results. This was investigated by examining the effects of a nutritional supplementation (GAKIC) on fatigue during high-intensity, repeated cycle sprints in trained cyclists.

2. To examine the application of established guidelines for food and fluid intake on an elite group of endurance runners. This was achieved by assessing the food and macronutrient intake of elite Ethiopian distance runners during a period of high-intensity exercise training at altitude and prior to major competition. The data were also compared to previous studies conducted on East African runners.

3. To describe the drinking behaviours of elite male marathon runners during major city marathons. The results from the current study allowed direct comparison to established guidelines on optimal hydration during long distance running.

4. To investigate the effects of a hyper-hydration strategy that combines Cr and Gly supplementation on thermoregulatory responses in hot and cool conditions. The data allowed examination of a possible method that will bring together the established guidelines and the data extracted from "real world" studies.

6.1 Commercial supplements and the industry role

Chapter 2 examined the issues associated with mixed commercial supplements as well as the lack of well-controlled studies conducted on trained subjects while focusing on a specific commercial supplement (GAKIC). Notably, the data from previous investigations (Buford & Koch, 2004; Stevens, et al., 2000) suggested that supplementation with GAKIC enhances peak power output and attenuates the decline in power output during repeated high intensity sprints (Buford & Koch, 2004; Stevens, et al., 2000). In the initial study, (Stevens, et al., 2000), it was demonstrated that GAKIC supplementation increased muscle force production by up to 28%, increased total muscle work by at least 12% and improved overall performance compared to Pl. The high intensity experimental protocol of Stevens,

et al., (2000), consisted of 35 maximal, isokinetic repetitions at a rate of $90°\cdot s^{-1}$ knee extensions. In the later study, Buford & Koch (2004) reported a significant difference in mean power output during high intensity exercise consisting of 5 repeated sprints of 10 s on a cycle ergometer. Repeated measures analysis of covariance indicated a significant difference between Pl vs. GAKIC in the pattern of change in mean power output over the five sprints. Buford & Koch (2004), using a *Post hoc* analysis revealed further that the decrease in mean power output between sprints 1 and 2 was significantly less with GAKIC than with Pl (-1 ± 9 W vs. -47 ± 18 W respectively). Furthermore, no differences were reported in the variables of peak power output or fatigue index between the two treatments. Although the experimental designs in the study by Buford and Koch (2004) as well as in the investigation presented in Chapter 2 were identical, the outcomes of the two studies were different. For instance, the well-trained male cyclists, who were studied in Chapter 2, completed two supra-maximal sprint tests each involving 10 sprints of 10 s separated by 50 s rest intervals on an electrically braked cycle ergometer. No differences were found in peak power between conditions which declined from the 1st sprint (Pl: 1332 ± 307 W, GAKIC: 1367 ± 342 W) to the 10th sprint (Pl: 1091 ± 229 W, GAKIC: 1061 ± 272 W). Mean power declined from the 1st sprint (Pl: 892 ± 151 W, GAKIC: 892 ± 153 W) to the 10th sprint (Pl: 766 ± 120 W, GAKIC: 752 ± 138 W) and did not differ between conditions. Fatigue index remained at ~38% throughout the series of sprints and did not differ between conditions. Therefore, GAKIC supplementation did not have an ergogenic effect on muscle power output or attenuation of fatigue during repeated sprints in trained cyclists and this conclusion is in contrast to previous investigations (Buford & Koch, 2004; Stevens, et al., 2000).

The data presented in Chapter 2 raise two main concerns. The first and most notable is the fact that findings of the previous studies (Buford & Koch, 2004; Stevens, et al., 2000) differ from the findings of the current thesis and this could be due to differences existing in the experimental design and methodology employed. The fact that trained athletes were employed in the present study (Chapter 2) while non-athletes took part in studies by Stevens et al. (2001) and Buford et al. (2004) may account for the existing discrepancies in findings. Indeed, as indicated in Figure 6.1, Buford and Koch (2004) found the smaller decline in power output for GAKIC compared with Pl between sprints 1 and 2. Notably, the values in sprint 1 vary between GAKIC and Pl treatments. This fact may be used as indicator regarding the training status of the participants. In general, the reliability of a performance test refers to the consistency or reproducibility of performance when someone

performs the test repeatedly (Hopkins, 2000). The first discrepancy in the methodology is the frequent exposure to high-intensity exercise on an almost daily basis, which may eliminate performance variability (Hopkins, et al., 2001). Secondly, physical conditioning during the course of a study should not change to the same extent in trained as in non-trained individuals. In the study by Buford and Koch (2004) untrained participants were utilised. Therefore, a systematic change (non-random change) in the mean value between 2 trials that applies to all study participants could be possible. This systematic change may be learning or training effect and is an important issue when volunteers perform a series of trials as part of a monitoring programme. Generally, untrained individuals perform the second trial better than the first, because they benefit from the experience of the first trial and learn how to do the test (Schabort, Hawley, Hopkins, & Blum, 1999). In tests of human performance, that may depend on effort or motivation, volunteers might also perform better in the second trial because they want to improve (Hopkins, 2000). Thirdly, in cases of trained participants, it is likely that the outcome of a human performance test is affected by small details an athlete is accustomed to or by practices adopted by each athlete (e.g., using customised cycling shoes and pedals, fixed cycling position etc.). Furthermore, the typical error between the experiment and reliability study becomes greater when differences in equipment, researchers, environment and characteristics of the volunteers are observed (Hopkins, 2000). Finally, the margin of error widens when non-trained athletes are utilized in a study, thus assuming a constant random error in absolute value rather than a constant error in percent value contributed by equipment or examiners. This error will have less impact on the overall outcome when expressed as a percentage of the higher power output of trained individuals (Hopkins, et al., 2001). Therefore, studies should minimize any learning effects (e.g., by utilizing trained-athletes) as well as get subjects to perform practice (or familiarisation) trials to reduce these effects. Thus, interpretation of the effectiveness of a study's outcome should be taken from tests with high reliability. In the present study (Chapter 2), variability in performance trials between familiarization trials was small (i.e., less than 5%) as would be expected from subjects who are well accustomed to the exercise protocols employed in the study (Coleman, et al., 2005; Hebestreit, et al., 1999; Hopkins, et al., 2001). The more stringent methodology used in the present study ensured that all possible confounding factors were minimised.

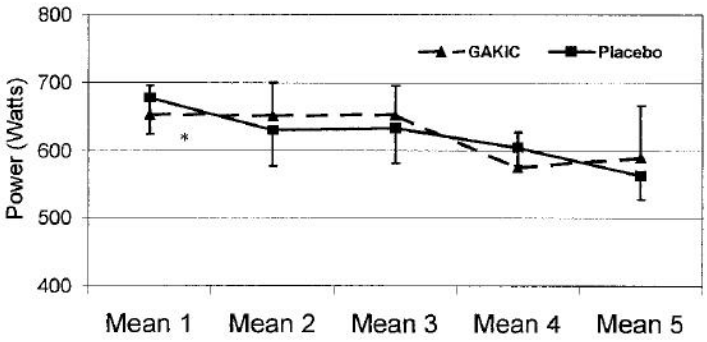


Figure 6.1 Mean power outputs over five repeated cycling sprints with GAKIC and placebo treatments (*N* = 10, mean ± SD). *Repeated measures ANCOVA indicated an overall significant difference (*P* = 0.039) between GAKIC and PI over the course of five sprints, with a significantly (*P* = 0.038) smaller decline in power output for GAKIC compared with PI between sprints 1 and 2. Adapted from Buford and Koch (2004).

As described in Chapter 1, the consumption of supplements is motivated by the pursuit of "performance enhancement". Athletes are constantly searching for new dietary supplements in an attempt to maximize athletic performance, to prevent diseases, to compensate for an inadequate diet and/or to overcome their own athletic limits (Alves & Lima, 2009). Consequently, the need to produce novel supplements stimulates the industry to manufacture "cocktails" consisting of mixed ingredients in an attempt to maximize the potential effect of the product thus making it more attractive to athletes (Green, et al., 2001). Additionally, the lack of strict regulations in supplement production can place the consumer at significant risk. DSHEA, which amended the Federal Food, Drug and Cosmetic Act, created a new regulatory framework for the safety and labelling of dietary supplements. Under this law, the manufacturer is responsible for determining that the dietary supplements it manufactures or distributes are safe (see also Chapter 1). Notably, Green at al. (2001) demonstrated this point clearly. These authors (Green, et al., 2001) analysed 12 brands of supplements by means of high-pressure liquid chromatography to identify the true ingredients in each package. The supplements contained 8 different steroids and were randomly selected for purchase in stores that supply nutritional products to athletes. Surprisingly, only 1 brand met the DSHEA labelling requirements (1994). 11 of 12 brands that did not meet the labelling requirements, contained less than the stated dose, 1 brand had 177% of the stated dose, and 2 brands contained none of a stated ingredient (Green, et al., 2001). Moreover, one brand contained 10 mg of testosterone, which, is an anabolic steroid, and its use is prohibited in the sporting arena (Green, et al., 2001). With the stringent rules adhered to by governing bodies of sports, this type of

contaminated supplement could cost the athlete immediate disqualification. The current study by Green et al (2001) verifies the concerns that the labelling of some "sports" nutritional supplements does not accurately reflect what is contained in the product. Therefore, athletes need to carefully consider even the use of "legal" nutritional supplements since manufacturing standards are not the same as pharmaceutical ones (Calfee & Fadale, 2006).

The modern idea of producing nutritional "cocktails" seems to be related with "GAKIC" supplementation. Surprisingly, when the Chapter 2 investigation was sent for review to the journal of Medicine and Science in Sports and Exercise, the following comments were received and clearly state that there is no rationale for combining the specific compounds of GAKIC. The following comments are notable to the study "To the opinion of this referee, there does not seem to be a clear physiological rationale for ingesting these specific compounds in combination. The study conceivably would have been much more interesting if the effects of the isolated components had been studied, alone or in combination." The justification for further investigation was to expand on why this specific combination was used in the supplement (with a specific dosage and time), and to base the findings on the previous studies. This interference of the commercial industry in the science of nutritional supplementation results in non-established outcomes, which is the second important conclusion of the investigation in Chapter 2. Therefore, it is imperative to make a connection between the marketing of supplements by the commercial industry and consumption by athletes. Indeed, it appears that "GAKIC" or "GAKIC HARDCORE" or "GAKIC Pro Series" as it is now known in the market (GAKIC, 2010), is the result of the industry's need to produce novel supplements. It should be noted that in both previous studies (which are referenced on the label of the supplement bottle) (Buford & Koch, 2004; Stevens, et al., 2000) it was concluded that the specific combination of amino- and keto-acids contained in GAKIC worked synergistically in order to improve high-intensity exercise performance through their relevant metabolic pathway(s). Nevertheless, both studies were unable to report any clear mechanism(s) by which the components of GAKIC acted in the way claimed by the manufacturer (individually or synergistically). Unfortunately, the supplement is on the market despite the fact that the instantaneous ergogenic effects claimed to be yielded by the product do not exist according to current evidence found in the investigation presented in Chapter 2. In the case of GAKIC, athletes may choose to consume this specific nutritional "aid" in order to enhance competitive performance and promote adaptations to training spending approximately $70 per month

(see also Table 1.1 for direct comparison with other nutritional supplements). Nevertheless, as mentioned in Chapter 1, before deciding to use a supplement, athletes should always consider the issues of efficacy, safety and legality/ethics associated with the product (Burke, et al., 2009), and decisions about effectiveness must be extrapolated from the best available research. After controlling the primary cofactors, it seems that GAKIC should be placed in the category of "apparently ineffective" supplements (Table 1.2) based on the data presented in Chapter 2.

6.2 Food and fluid intake of elite endurance runners

Chapters 3-4 provided a rare insight into the lifestyle, training practices, and food and fluid intake strategies of some of the most successful endurance runners in the world. Both investigations in these chapters also aimed to assess the food and fluid practices of elite endurance runners and compare them to previous studies, as well as to the current recommendations for endurance athletes. A number of studies have reported the dietary intake and energy balance status of elite African endurance runners (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Mukeshi & Thairu, 1993; Onywera, et al., 2004). Elite East African runners may be considered as an appropriate target group for conducting investigations, because they dominate endurance running (Fudge, et al., 2008; Onywera, et al., 2004; Scott & Pitsiladis, 2007). In the first of these studies, Mukeshi and Thairu (Mukeshi & Thairu, 1993) estimated the EI of male, long distance Kenyan runners through a combination of questionnaires and direct observation. Remarkably low EI (9790 $kJ \cdot day^{-1}$ on average) was reported, while the average CHO intake was 441 g (8.1 $g \cdot kg^{-1}$ of BM per day) or 75% of TEI. However, in subsequent studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004), substantially higher estimates of EI were noted in comparison to the initial data. For example, Christensen et al. (2002) reported an average EI of 13210 $kJ \cdot day^{-1}$, while the consumption of CHO was 476 g (8.7 $g \cdot kg^{-1}$ BM, 71% of TEI). Similarly, Onywera et al. (2004) reported an average EI of 12486 $kJ \cdot day^{-1}$ (CHO 607 g, 10.4 $g \cdot kg^{-1}$ BM and 76.5% TEI), while estimated EI in two studies by Fudge and colleagues was 13241 $kJ \cdot day^{-1}$ (CHO 552 g, 9.8 $g \cdot kg^{-1}$ BM and 71% TEI) (Fudge, et al., 2006b) and 12300 $kJ \cdot day^{-1}$ (CHO 580 g, 9.8 $g \cdot kg^{-1}$ BM, 79% TEI) (Fudge, et al., 2008), respectively. Chapter 3 contains the only investigation which has been currently conducted on elite Ethiopian endurance runners. It was found that the athletes studied, met macronutrient dietary recommendations and guidelines for endurance athletes (Rodriguez, et al., 2009), but did not meet the guidelines for fluid intake (Sawka,

et al., 2007). For instance, total average EI was 13375 ± 1378 kJ and is in agreement with previous studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004) (~ 12809 kJ·day^{-1} on average). Furthermore, macronutrient intake of Ethiopian long distance runners fulfilled recent recommendations (Rodriguez, et al., 2009). CHO intake was 64.3% TEI (9.7 g·kg^{-1} per day) and the daily CHO intake was 545 ± 49 g (Figure 3.2, Figure 3.3), while recommendations for male and female athletes range between 6 to 10 g·kg^{-1} of BM per day (Rodriguez, et al., 2009). These results are also in agreement with previous studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Mukeshi & Thairu, 1993; Onywera, et al., 2004) when the daily amount of CHO was well above 65% of TEI, ranging from 8.1 to 10.4 g·kg^{-1} BM. Protein intake was 12.4% of TEI (Figure 3.2, Figure 3.3) (1.76 g·kg^{-1} BM per day with a daily intake of 99 ± 13 g) of which 76% was delivered from vegetable sources (Table 3.3) and well within the current recommendations for endurance athletes (1.2 to 1.7 g·kg^{-1} BM per day) (Rodriguez, et al., 2009). This is also in agreement with the literature (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004) where daily protein intake ranged from 1.3 to 2.2 g·kg^{-1} BM. Although, the values from Chapter 3 (23.3% TEI, Figure 3.2) for fat intake were in agreement with the guidelines (Rodriguez, et al., 2009), they were somewhat higher in comparison to values (6.6 to 17.4% of TEI) observed in previous investigations (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Mukeshi & Thairu, 1993; Onywera, et al., 2004). Additionally, results from Chapter 3 corroborate the findings of previous studies (Christensen, et al., 2002; Fudge, et al., 2008; Fudge, et al., 2006b; Mukeshi & Thairu, 1993; Onywera, et al., 2004) regarding fluid intake. In fact, the athletes consumed approximately 1.75 L·day^{-1} of fluids which comprised mainly of water and athletes did not in general consume water before or during training; on some occasions small amounts of water were consumed following training. Onywera and colleagues (Onywera, et al., 2004) reported a modest fluid consumption (2.3 L·day^{-1}). Similar fluid intake (1.8 L·day^{-1}) was observed by Fudge et al. (Fudge, et al., 2006b) and in a subsequent study by the same research group (2.3 L·day^{-1}) (Fudge, et al., 2008). These studies collectively demonstrate that elite East African endurance runners do not consume any fluids before or during training, whilst modest amounts of fluids are consumed after training and only by a small number of runners (Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004). According to the current recommendations (Sawka, et al., 2007) athletes are advised to consume adequate amounts of beverages in order to prevent significant dehydration (> 2% BM loss from water deficit) and excessive changes in electrolyte balance. As a consequence, athletes are recommended to consume ~5–7

$mL \cdot kg^{-1}$ BM at least 4 h before the exercise task, while a 0.4 to 0.8 $L \cdot h^{-1}$ (*ad libitum*) range is suggested during exercise. ACSM prevailing guidelines (Sawka, et al., 2007) advise athletes to drink ~ 1.5 L of fluid for each kg of BM lost. Therefore, the amount of fluids (as dietary water intake) consumed by the subjects of the study presented in Chapter 3 would be inadequate to maintain the hydration status of the athletes.

To supplement the investigation of Chapter 3 regarding the drinking behaviours of successful elite marathon runners and in the process assess the efficacy of the current fluid intake recommendations, a retrospective analysis of the drinking behaviours of 10 male athletes during 13 successful city marathons was undertaken using available video footage (Chapter 4). It should be noted that there is almost no information on what the best marathoners drink when racing (for a review of the marathon running literature see reference (Cheuvront & Haymes, 2001b). The ambient conditions during the 13 studied marathon races were 15.3 ± 8.6 ^{o}C (ambient temperature) and 59 ± 17% relative humidity; average marathon competition time was 02:06:31 ± 00:01:08 (h:min:s). In fact, the data of the investigation in Chapter 4 demonstrate a wide range of individual rates of fluid ingestion by the elite runners during major city marathons (Figure 4.2). Notably, no significant correlations were found between total drink duration, ambient temperature ($r < 0.1$, $P = 0.77$; Figure 6.2) and relative humidity ($r < 0.5$, $P = 0.12$; Figure 6.3) of the marathon races. Warmer conditions did not always result in greater total drinking durations. For example, during the London 2006 marathon the winning athlete consumed more fluid than during the London 2007 marathon (2290 vs. 1400 mL) even though the 2006 race was run in cooler conditions (10 vs. 18 °C) (Table 4.1). Similarly, the runner in the New York 2008 marathon consumed less fluid (73 mL) than did the winner of the Fukuoka 2009 marathon (1458 mL) even though both races were run at almost identical conditions (6 vs. 7 °C, respectively). The second significant finding of the study in Chapter 4 is that the fluid intake rates of elite runners (0.55 ± 0.34 $L \cdot h^{-1}$) are within the current *ad libitum* fluid intake recommendations of 0.4 – 0.8 $L \cdot h^{-1}$ initially proposed by IMMDA (Noakes, 2003a) and subsequently adopted by the ACSM (Sawka, et al., 2007). However, physical data collected during the Dubai 2009 marathon showed that the athlete, who set the previous world marathon record in 2008, finished the race successfully with a reduction in BM of 5.7 kg equivalent to a 9.8% BM reduction. Notably, the fluid intake rates (> 0.8 $L \cdot h^{-1}$) of the runner exceeded the upper limit of the current guidelines, while his estimated sweat rate was approximately 3.6 $L \cdot h^{-1}$. Many investigations (Muir, et al., 1970; Pugh, et al., 1967; Zouhal, et al., 2010) have also reported that BM losses > 3-4% are

common in faster finishing competitors. Additionally, there appears to be a weak inverse relationship between BM loss and athletic performance, leading to the conclusion that the fastest finishers usually lose the most weight. Pugh et al. (1967) examined marathon runners by measuring their BM and T_{core} during a marathon race under mild enviromental conditions (23 °C ambient temperature and 58% relative humidity) and found that the winner's BM loss was 5.2 kg (6.7% BM). Those authors (Pugh, et al., 1967) also stated that a further condition of success seems to be a high tolerance of fluid loss. Particularly notable is the fact that the majority of those who failed to finish the event had lower weight losses (Pugh, et al., 1967). Therefore, as a consequence of these very high sweat rates, recommended fluid ingestion rates seem inadequate for the prevention of significant sweat/BM loss in order to maintain athlete's hydration status, during elite marathon running.

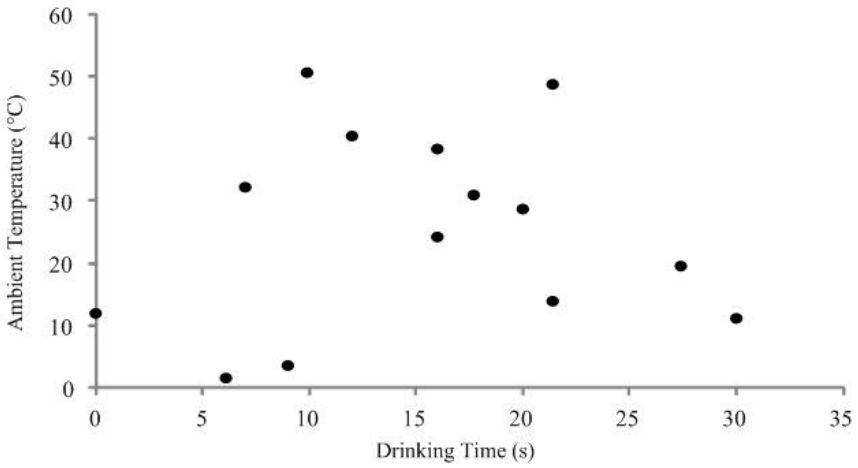


Figure 6.2 A plot of ambient temperature (°C) of all marathon races against total time consuming fluid (in seconds) during the respective marathon.

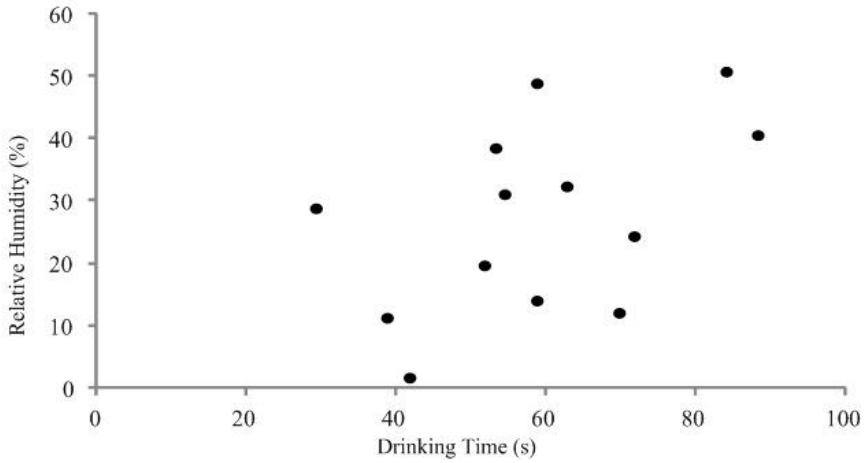


Figure 6.3 A plot of relative humidity (%) of all marathon races against total time consuming fluid (in seconds) during the respective marathon.

In Chapter 4 it was concluded that, elite athletes do not seem to maintain their BM during successful marathon racing within current recommended ranges of 2 – 3%. However, a number of limitations regarding the aforementioned Chapter should be stated. The most obvious limitation of the data presented in Chapter 4 is the retrospective survey study design involving the analysis of video footage in order to estimate fluid consumption of elite runners during marathon racing. Inevitably, this indirect approach resulted in incomplete/missing data as the drinking behaviours of the main protagonists (e.g., race leaders) were at times not visible in the footage due to technical problems, drinking being obscured by other runners in close proximity, or no footage of a drinking station. As such, some 72% (ranging from 33% for Athens 2008 to 100% London 2007) of all drinking was observed on the video footage and therefore incomplete/missing data was extrapolated from the observed data (Table 4.2). The indirect approach used in the present study did not allow the composition of the athletes' drink to be determined. Furthermore, the lack of information about pre-race hydration or CHO status is a further limitation in the attempt of assessing the hydration status of the runners. The average flow rate (i.e., 45.2 $mL \cdot s^{-1}$) for standard sport drink bottles used on the present analysis was determined from a supplementary laboratory drinking simulation experiment conducted in recreationally active individuals and not elite runners. There is also an increasing amount of evidence accumulating questioning the accuracy of using BM losses during exercise as a surrogate marker for changes in TBW (Nolte, Noakes, & van Vuuren, 2011). In the study presented in Chapter 4, the change in BM was calculated as the difference between the BM measured immediately before and after exercise (i.e., acclimation experiments and the Dubai

marathon). As such, the delta BM-derived TBW changes are certainly overestimated as the production of metabolic water from fuel oxidation and water released from muscle and liver glycogen breakdown are not taken into account (Maughan, Shirreffs & Leiper, 2007). This portion of released water from glycogen oxidation during exercise varies among athletes depending on the glycogen storage. For instance, a typical East African diet, rich in CHO, allows the optimal storage of liver and muscle glycogen. This is in contrast to what appears to be the case for athletes from industrialised countries, where CHO intake can fall at the lower end of the range recommended for endurance athletes (e.g., 6.1 $g \cdot kg^{-1}$ BM per day; Moses & Manore, 1991), especially when a typically Western diet of 55–58% CHO (ACSM, 2000) is consumed. Interestingly, it has been calculated that an athlete who loses 2 kg of BM during a marathon race would be dehydrated as low as approximately 200 g when allowance is made for the weight lost due to the production of metabolic water and by the release of water stored in glycogen complexes in muscle and liver (Pastene, Germain, Allevard, Gharib & Lacour, 1996). Despite the limitation of BM-derived sweat loss estimation, similar measurements would have been desirable in all studied elite runners but proved impossible. Similarly, direct measurement of TBW losses using the diluted isotope method (e.g., deuterium oxide) was impossible given the study constraints and therefore the term "dehydration" with associated ergolytic effects is not used to describe the marathoners. The present analysis is in conjunction with the limited available literature (Daries, et al., 2000) provide reasonable evidence that elite marathon runners can perform well despite significant BM loss. Despite these important limitations, the novel approaches adopted in this analysis have allowed meaningful conclusions with regard to the drinking practices of elite marathoners during competition to be determined.

The evidence presented in the current thesis (Chapter 3-4) is not the only reason that renders the current recommendations questionable. "Real world" evidence exists that contradicts the prevailing guidelines. For example, Emile Zatopek (1952 Helsinki Olympic Marathon), Jim Peters (Multiple World Marathon Records 1952-1954), Abebe Bikila (1960 and 1964 Olympic Marathons), Ron Hill (1970 Edinburgh Empire Games) are all runners who did not drink during successful marathon races. Generally, it has been shown that athletes who lose the most BM during marathon and ultra-marathon races as well as ironman triathlons are usually the most successful (Cheuvront & Haymes, 2001b; Muir, et al., 1970; Pugh, et al., 1967; Sharwood, et al., 2004; Wharam, et al., 2006; Wyndham & Strydom, 1969; Zouhal, et al., 2010; Zouhal, et al., 2009). Additionally, Zouhal et al. (2010) studied 643 marathon finishers during the 2009 Mont Saint-Michel

Marathon in France. These authors found BM loss during the marathon to be inversely related to race finishing time in 643 marathon runners and was > 3% in runners completing the race in less than 3 h (Zouhal, et al., 2010). It should be noted that the degree of BM change varied from a loss of 8% to a gain of 5%. This range of weight change (-8 to +5% BM; Figure 6.4) occurred even though all runners received the same pre-race advice and had the same access to fluid during the race. Consequently, the question has been raised as to why so many athletes would choose to lose more than 3% BM when enough fluid was available to allow some to gain 5% BM (Zouhal, et al., 2010). The practical implementation of the nutritional guidelines has been questioned (Noakes, 2007a, 2007b; Zouhal, et al., 2010), because it seems impossible to make recommendations for all situations. Individuals in different climatic conditions with different fluid losses, different modes, durations, and intensities of exercise need to be considered. It is noted that dehydration results from all sweat, respiration, urine, and insensible skin losses (Casa, Clarkson, & Roberts, 2005). Additionally, there is no evidence that fluid intake during exercise increases sweat rates, stroke volume, or reduces overall cardiovascular strain (i.e., increase HR and reduce stroke volume, cardiac output and mean arterial pressure). Furthermore, fluid intake during exercise alters skin and muscle blood flow, increases oxygen, and substrate delivery to the working muscle (Fudge, et al., 2006a). Moreover, Noakes et al. (1991) support the suggestion that heat stroke can occur in hot conditions without dehydration if the heat from muscle work cannot be removed from the body at a rate sufficient to prevent a progressive rise in T_{core} (Casa, et al., 2005). This view of the drinking behaviours (i.e., *ad libitum*) of elite endurance runners is corroborated by the investigations presented in Chapters 3-4, as in previous studies (Fudge, et al., 2008; Fudge, et al., 2006b; Onywera, et al., 2004).

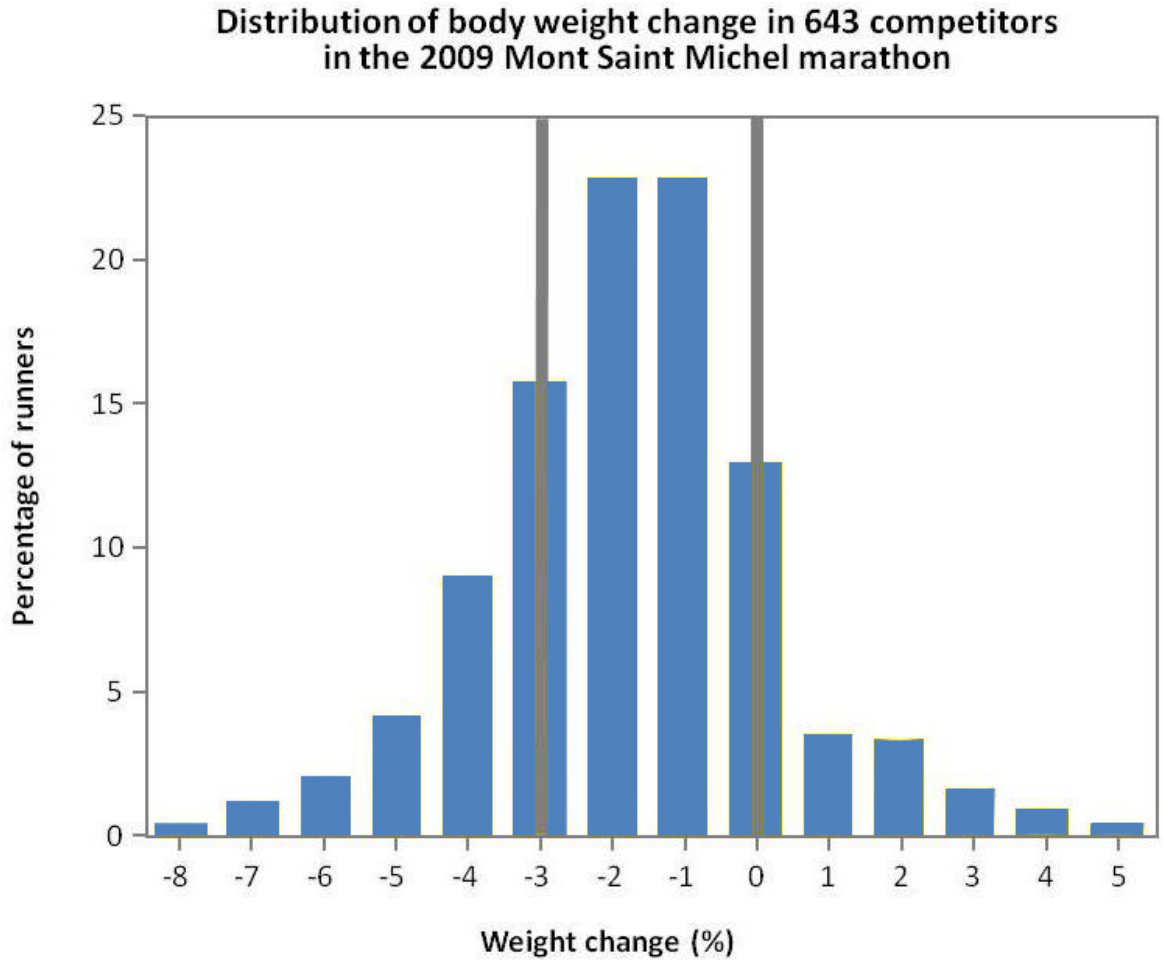


Figure 6.4 Distribution of body weight change in 643 competitors in the 2009 Mont Saint Michel marathon. Adapted from Zouhal et al. (2010).

The reduction of the energy cost of movement at a sub-maximal velocity by way of reducing BM to improve running performance is well known (Cureton & Sparling, 1980; Cureton et al., 1978; Marino et al., 2000; Myers & Steudel, 1985). When humans or animals carry extra mass while running or walking, the energy cost per unit distance increases in direct proportion to the additional load which is expressed as a percentage of the BM according to Taylor et al. (1980). In fact, Wyndham and Strydom (1969) reported no correlation with percent dehydration and T_{core} until a 3% weight deficit had occurred. Contrastingly, the study by Armstrong et al. (2006) showed that $\dot{V}O_2$ (expressed relative to BM) was not significantly influenced by a loss in BM as high as 5.7% (Armstrong, et al., 2006). On the other hand, the widely adopted *ad libitum* strategy during marathon racing seems to produce optimal/winning performances. Supported by this evidence are the findings regarding the marathon runner who set the previous world record in 2008 and finished a competitive race (Dubai, 2009) with a BM loss of 9.8%. This evidence would

suggest that there exists a tolerable range for dehydration that may not negatively impact running performance. Other studies (Muir, et al., 1970; Pugh, et al., 1967; Zouhal, et al., 2010) can also confirm this notion since a range of 3-4% seems to be common in faster finishing competitors. The evidence may even confer an advantage by preventing a significant increase in BM due to the "over-consumption" of large volumes of fluid. Here a "drink only when you are thirsty" fluid intake strategy (Chapters 3-4) may yield to a balance between drinking enough fluid to maintain an optimal zone of tolerable dehydration and reducing the absolute energy cost of movement by reducing the athletes' BM.

6.3 Hydration and running economy

In 2007, ACSM replaced their prior Position Stand (Convertino, et al., 1996) with an updated one on exercise and fluid replacement (Sawka, et al., 2007) that advocates drinking *ad libitum* (0.4 - 0.8 $L \cdot h^{-1}$) during exercise (with the lower value for slower, lighter individuals competing in cooler environments, and the higher value for faster, larger individuals competing in warmer environments) in order to prevent excessive dehydration (i.e., < 2% BM loss). By comparison, when given the data extracted from the investigation in Chapter 4 regarding the extremely high values of body water deficit during a race, (i.e., ~10% BW loss) an alternative hydrating strategy, rather than the widely accepted fluid intake method, is warranted in order to bring together the established guidelines and data extracted from "real world" studies (e.g., Chapter 4). The strategy investigated in Chapter 5 may expand the body's water compartments, maintain PV, and consequently attenuate the rise in T_{core} and HR during exercise--especially in hot and humid environments as well as being practical and direct in order to be followed by elite athletes. This alternative hydrating strategy was achieved by means of a pre-exercise water loading strategy with use of hydrating agents (i.e., Cr and Gly) (Magal, et al., 2003; Riedesel, et al., 1987) in combination (Easton, et al., 2007).

Hydration status appears to be a critical factor for temperature and cardiovascular regulation during exercise in the heat (Gonzalez-Alonso, et al., 1995; Nadel, et al., 1980). In the investigation presented in Chapter 5, combined Cr and Gly supplementation was administered to 15 male endurance runners during a 7-day period. Exercise trials were conducted pre- and post-supplementation at 10 and 35 °C and 70% relative humidity. BM and TBW increased by 0.90 kg and 0.71 L, respectively following supplementation. Despite the significant increase in BM, supplementation had no effect on $\dot{V}O_2$ and

therefore RE. Both HR and T_{core} were attenuated significantly during exercise after supplementation. Nevertheless, TC and RPE did not significantly differ between pre- and post-supplementation. Similarly, no significant differences were found in PV pre- and post-supplementation despite the increase in TBW, ICW and ECW. Considering the aforementioned and the fact that free water in the form of sweat is mainly lost from the plasma, it can be suggested that the increase in other water compartments resulted in water moving towards the plasma due to an osmotic gradient. This in turn leaves the PV unaffected. It should also be noted that in order for blood volume to be maintained in cases of sweating, fluid loss is obtained in varying proportions from ECW as well as ICW compartments of the body (Sawka, et al., 2001; Verbalis, 2003) (Figure 6.5). Furthermore, it would be expected that as body water loss increases through sweating, there would be an increase in T_{core} during exercise in the heat (Sawka, et al., 2001). Therefore, increasing the body water would result in maintaining T_{core} during exercise in the heat. In the study present in Chapter 5, Cr and Gly induced an increase in ICW and consequently there was a significant attenuation in the rise of T_{core} by 0.28 °C during exercise in the heat (Figure 5.7). It is possible that ICW was raised by Cr and Gly supplementation which, as a consequence, resulted in an increase of the specific heat capacity of the body (Kilduff, et al., 2004).

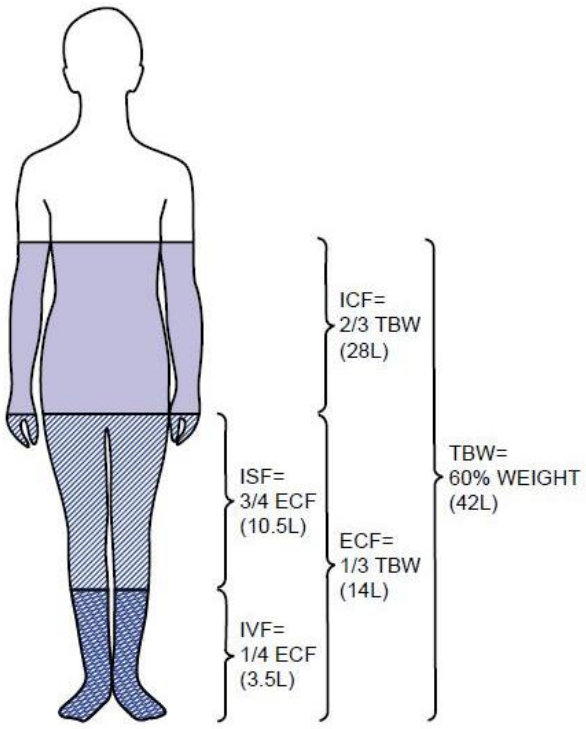


Figure 6.5 Schematic representation of body fluid compartments in man. The shaded areas depict the approximate size of each compartment as a function of body mass. The figures indicate the relative sizes of the various fluid compartments and the approximate absolute

volumes of the compartments (in liters) in a 70-kg adult. TBW = total body water; ICF = intracellular fluid; ECF = extracellular fluid; ISF = interstitial fluid; IVF = intravascular fluid. The arrows indicated the direction of fluid shift during sweat loss. Adapted from Verbalis (2003).

Supplementation with combined hydrating agents such as Gly or Cr has consistently produced modest fluid retention of 400-800 mL (Kern, et al., 2001; Magal, et al., 2003; Riedesel, et al., 1987). Although the combination of the hyper-hydrating agents previously mentioned results in an increase in TBW, and hence in a reduction in certain cardiovascular and thermoregulatory responses (Easton, et al., 2007), the increase in hydration status due to an increase in BM may be expected to increase RE as previously described in Chapter 5. Nevertheless, the data from the current investigation in Chapter 5 indicated that this increase in BM had no negative effect on trained endurance runners' RE at 60% $\dot{V}O_2$max, therefore, it would suggest that a pre-exercise hyper-hydration method may be ideal for optimal hydration on endurance runners. The present data suggest that an increase in BM of about 1.4% (i.e., average increase in BM in the present study) has no detrimental effect on $\dot{V}O_2$ and consequently (at least theoretically) on exercise performance. It was found that a pre-exercise water loading strategy using a combination of hydrating agents such as Cr and Gly resulted in a significant increase in body water compartments, which reduced cardiovascular (Figure 5.4) and thermoregulatory (Figure 5.7) responses during running at 35 ^{o}C and had no impact on the oxygen cost of running and thus the RE of runners at 60% $\dot{V}O_2$max.

6.4 Future directions

Chapter 2 demonstrated that a specific combination of amino and keto acids contained in GAKIC supplementation did not act as an ergogenic aid. Nevertheless, a future study of the effects of the isolated components, alone or in combination may be interesting for further research.

Chapters 2 and 3 reported that elite endurance runners did not consume the recommended amounts of fluids before, during and after training, as well as during running in the most prestigious marathon races. Nevertheless, a deeper insight into the hydration status with direct measurements on sweat loss and body water compartments would be beneficial to the literature as there is a need for novel data that can be obtained from some of the best

athletes in the world in order to assess and possibly improve the applicability of the current recommendations to elite athletes.

Given the association between attainment of a critical T_{core} and the development of fatigue and taking into consideration that hyper-hydration induced by Cr and Gly supplementation attenuated increase in T_{core} and had no impact on the oxygen costs of running, it is tempting to speculate that the hyper-hydration strategy used in this study may be expected to increase exercise performance. However, this needs to be clarified by future studies, by further examining how the positive effects of cardiovascular and thermoregulatory responses can impair exercise performance.

As suggested in Chapter 1, the methodology above (Chapter 5) should be applied to elite athletes in "real world" conditions to extract accurate data. The guidelines for optimal hydration status and the practical application of the guidelines have to be researched further.

6.5 General Conclusions

Chapter 1 explores the factors which affect exercise performance. Chapter 1 also raises the issue of difficulty to establish well acceptable guidelines. Additionally, it demonstrates the need for evidence based data on well-trained and elite athletes. Therefore Chapter 2 focuses on a specific nutritional supplement (GAKIC) in order to compare the data with previous studies using non-trained athletes as well as to expose potential issues associated with the commercial industry and sports enhancing supplements. Chapter 3 and 4 provide a rare insight into the lifestyle, training practices, and food and fluid intake strategies of some of the most successful endurance runners in the world. The results of the research allow direct comparison with the established guidelines for food and fluid intake for endurance athletes as well as with previous studies conducted on elite runners. Chapter 5 provides a possible method that could bring together the established guidelines regarding the hydration status during running and the data extracted from "real world" studies. The main findings are as follows:

1. Chapter 2 reported that GAKIC supplementation does not enhance high intensity exercise performance. After controlling for possible confounding factors that could have adversely affected the results, it was found that GAKIC supplementation had no effect on peak power, mean power or fatigue index, in contrast to the findings of previous studies

(Buford & Koch, 2004; Stevens, et al., 2000). On the basis of these findings and those of previous studies involving the individual components of GAKIC, there appears little evidence to support the ergogenic effects of GAKIC during high intensity exercise performance when applied to well-trained subjects.

2. Chapter 3 found that elite Ethiopian runners met dietary recommendations for endurance athletes for macronutrient intake but not for fluid intake. These data are in accordance with previous studies conducted on elite Kenyan endurance runners.

3. Chapter 4 found that the estimated fluid intake rates of the winners during the 13 races reported in the investigation (Table 4.2) are broadly within current recommendations (Sawka, et al., 2007). However, these elite runners do not seem to maintain their BM during successful marathon racing within current recommended ranges of 2 – 3%. On the other hand, this apparently widely adopted *ad libitum* strategy during marathon racing seems to produce optimal/winning performances. This evidence and the finding that the runner, who set the previous world marathon record in 2008, finished a competitive race (Dubai, 2009) with a BM loss of 9.8%, would suggest that there exists a tolerable range for dehydration that may not negatively impact on running performance, but may even confer an advantage by preventing a significant increase in BM due to the "over - consumption" of large volumes of fluid. This finding is in accordance with a recent meta – analysis which suggests that *ad libitum* drinking produces optimum performance (Goulet & Dugas, 2010).

4. Chapter 5 found that supplementation with combined Cr and Gly for 7 days was effective in increasing TBW and reducing cardiovascular and thermal strain during prolonged exercise in the heat. Despite the increased hydration resulting in a higher BM associated with combined Cr and Gly, there were no changes in runners' energy demands. These results are in accordance with a previous study by Easton et al. (2007). Hyper-hydrating before exercise through Cr and Gly seems to be a key for maintaining body euhydration for longer. Finally, no measures were taken that would enable the potential physiological advantage induced reductions in HR and T_{core} to be quantified.

Appendices

Appendix A: Subject Information Sheet

University of Glasgow
Institute of Biomedical and Life Sciences
University of Glasgow

INFORMATION SHEET

Study title: The effects of glycine-arginine-[alpha]-ketoisocaproic acid on fatigue during repeated cycle sprints in trained humans.

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study? We wish to find out whether taking certain previously used (by athletes) substances may increase your ability to work hard during cycle sprints, by reducing ammonia levels that intense exercise induces. We will measure your ability to perform strenuous exercise lasting approximately 10 s. The substances you will be required to ingest orally are glycine-arginine-[alpha]-ketoisocaproic acid (GAKIC) which is commercially available over the counter from most health shops. This amino-acid supplement is popular amongst athletes as it is thought to improve high intensity exercise performance especially when repeated bouts of exercise are required (e.g., weight lifting).

Why have I been chosen? You have been selected as a possible participant in this investigation because you regularly take part in endurance activity and you are in good health. Ten volunteers are being sought.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent

form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part? You will be asked to visit the laboratory on three occasions over a three-week period (see Table). All tests will last approximately 1 h. You will be familiarised to the cycle test during the first visit to the laboratory. On the first experimental cycle test you will consume either 10 g of GAKIC or 10 g of sucrose 45 min before the test (to be consumed with fruit juice). The recommended daily allowance of protein and amino acid in the diet is 56 g per day. For the final week you will take the opposite supplement to what you were took initially. So, if you initially took GAKIC, you will then change to the placebo supplement. You will not know which group you are in, until all the tests have been completed. The protocol for the second supplement is the same as the first. On your first visit to the lab you will be asked to complete two confidential questionnaires; the first will allow us to obtain information related to your general health; and the second will allow us to quantify your past exercise/activity involvement.

Your heart rate (HR) will be measured throughout exercise using a Polar HR monitor strapped round the chest. During each sprint we will monitor power output throughout. Your height, weight and percentage body fat will also be measured on the first visit to the lab. Your percentage body fat will be estimated by a bioelectrical impedance technique, which involves placing slightly adhesive small patches ("electrodes") on your right hand and foot and introducing a very small and imperceptible electrical current between these.

In order to estimate your nutritional intake, we will ask you to record your normal food and drink intake for 24-h prior to the test.

Finally, you will not be able to consume any alcohol 48-h prior to each lab visit. You will be excluded from participating in this study if you take drugs (recreational or performance enhancing drugs).

What are the side effects of taking part? There have been no reported side effects of GAKIC ingestion.

What are the possible disadvantages and risks of taking part? Exercise has a negligible risk in healthy adults, although maximal exercise has a small risk of inducing

myocardial ischaemia ("heart attack"). The primary symptom of myocardial ischaemia is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately. Some subjects experience mild discomfort from prolonged sitting on the seat of the cycle ergometer.

What are the possible benefits of taking part? We hope that you will find out more about how your body responds to supplementation with GAKIC and subsequent strenuous exercise. This information may help us better understand the mechanisms associated with muscle fatigue during strenuous exercise.

What if something goes wrong? If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified are fully trained in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff is on hand. You may want to consult your GP if you are experiencing any side effects from taking part in the study and should also inform the Principal Investigator.

Will my taking part in this study be kept confidential? All information about you that is collected during the course of the research will be kept strictly confidential

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication**.**

Table: Schedule of visits and proposed tests.

Visit	Test	Duration (hrs)
1	**Familiarisation and baseline measurements**	**1**
2	**Performance Cycle Test**	**1**
3	**Performance Cycle Test**	**1**

If you wish to find out more about this investigation, you can contact:

Dr Yannis Pitsiladis

Lecturer, Institute of Biomedical and Life Sciences

West Medical Building

University of Glasgow

Glasgow, G12 8QQ

Phone: 0141 330 3858

Fax: 0141 330 6542

e-mail: Y.Pitsiladis@bio.gla.ac.uk

Consent Form

I ..... give my consent to the research procedures which are outlined above, the aim, procedures and possible consequences of which have been outlined to me

Signature ……

Date ……

Appendix B: Subject Information Sheet

University of Glasgow
Faculty of Biomedical & Life Sciences

INFORMATION SHEET

Study Title: The effects of hyperhydration on running economy of endurance trained runners.

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study? We wish to find out whether taking certain substances (previously used by athletes) which increase the volume of water in your body may increase the amount of energy you use when running but help you to stay cooler in the process. We will measure your body water, blood volume and weight before and after exercise and your heart rate (HR), core temperature (T_{core}) and expired gas while running. The substances you will be required to ingest orally are creatine (Cr; a food element found in high abundance in meat and fish but also made by the body), glycerol (Gly; another substance found naturally in the body, which contributes to making energy for exercise). Cr in its pure form is commercially available over the counter from most health shops. This supplement is popular amongst athletes as it is thought to improve high intensity exercise performance especially when repeated bouts of exercise are required (e.g. football, rugby).

Why have I been chosen? You have been selected as a possible participant in this investigation because you regularly take part in endurance activity and you are in good health. Twelve volunteers are being sought.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form and fill in a lifestyle questionnaire. If you decide to take part you are still free to withdraw at any time and without giving reason.

What will happen to me if I take part? You will be asked to visit the laboratory on four occasions and a running track once, where a series of assessments will be carried out. All experimental trials should last no longer than 2 h. On your initial visit to the laboratory you will be medically examined by a qualified doctor. The first test will take place in the laboratory on a motorised treadmill at a fixed 1% gradient. During this test you will be instructed to run at 10, 11, 12, 13, 14, 15, 16, 17 and 18 $km \cdot h^{-1}$ (or until volitional exhaustion) for 3 minutes at each speed, with a 3-5 min rest interval between each bout walking at 4 $km \cdot h^{-1}$. The second test is exactly the same, except it is conducted outdoors on a 400m running track; an investigator will cycle next to you to monitor and control the running speed. The final four tests will take pace in the laboratory on a motorised treadmill; you will be required to run at a constant pace equating to 60% of your maximal capacity measured in the first test, for 30 min at 10 ^{o}C and 70% humidity. You will then receive a 30 min rest period before running again on the treadmill at the same speed as before but at 35 ^{o}C and 70% humidity. After the first 5 tests you will consume 2 L of experimental drink per day for 6 days and 1 L 5-h before your final test. The drinks will contain 10 g of Cr and Gly (0.75 $g \cdot kg^{-1}$ BM) dissolved in one litre of warm to hot water and flavoured with sugar free fruit juice.

HR and expired gas will be recorded throughout all tests via a HR monitor and a flexible rubber mouthpiece, respectively. You will also wear an accelerometer whilst running. In addition, you will be required to swallow a harmless recording device (a small pill like object) the evening prior to your test. This will enable the research team to monitor body temperature; there is no risk involved in swallowing the pill. All tests must be conducted at approximately the same time of day, and you will be required to wear the same athletic clothing/footwear for each test. During the final four tests we would like to take a small amount of blood from an intravenous line in the back of your hand. Your height, weight and percentage body fat will also be measured on each visit to the lab. Your percentage body fat will be estimated by a bioelectrical impedance technique, which involves placing slightly adhesive small patches ("electrodes") on your right hand and foot and introducing a very small and imperceptible electrical current between these.

In order to estimate your nutritional intake and energy expenditure, we may ask you to record your normal food and drink intake for 24-h prior to each test and to keep a diary of your physical activity.

Finally, you will not be able to perform any exercise 24-h prior to testing or consume any alcohol 48-h prior to each lab visit. You will be excluded from participating in this study if you take drugs (recreational or performance enhancing drugs).

What are the side effects of taking part? Some subjects have previously reported slight nausea, gastrointestinal distress (i.e., diarrhoea) and headaches. The only known 'side effect' of oral Cr supplementation that has been reported is a temporary increase in body weight. This increase in body weight is mostly due to an increase in water retention, not fat.

What are the possible disadvantages and risks of taking part? Exercise has a negligible risk in healthy adults, although maximal exercise has a small risk of myocardial infarction ("heart attack"). The primary symptom of myocardial infarction is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately. Intravenous lines through which blood is collected, may cause some bruising and subsequent soreness over the site of puncture and, rarely, a small wound (1-2 mm at most), which takes a few days to heal.

You will breathe through a rubber mouthpiece during the tests, in order for us to collect the air you breathe out. This is similar to the equipment used for snorkelling. You will also wear a nose clip. You may experience difficulty swallowing while breathing through a mouthpiece and wearing a nose clip, due to some pressure in the ears. In addition some subjects experience increased salvation when breathing through a mouthpiece.

What are the possible benefits of taking part? We hope to find out more about how your body responds to physical exercise after hyper-hydration. This information will help us to decide whether hyper-hydration should be recommended for those participating in running training or competition in a hot environment.

What if something goes wrong? If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it.

The principal investigators, although not medically qualified are fully trained in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff is on hand. You may want to consult your GP if you are experiencing any side effects from taking part in the study and should also inform the principal investigator.

Will my taking part in this study be kept confidential? All information about you that is collected during the course of the research will be kept strictly confidential.

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

If you wish to find out more about this investigation, you can contact:

Dr Yannis P. Pitsiladis
Institute of Biomedical & Life Sciences
West Medical Building
University of Glasgow
Glasgow, G12 8QQ

Phone: 0141-330-3858
Fax: 0141-330-2915
E-mail: Y.Pitsiladis@bio.gla.ac.uk

CONSENT

Title of Investigation: The effects of hyper-hydration on running economy of endurance trained runners.

I ……..

Give my consent to the research procedures which are outlined above, the aim, procedures and possible consequences of which have been outlined to me.

Signature **…….....**

Date **…….....**

Appendix C: Ethics Document

UNIVERSITY OF GLASGOW
FACULTY OF BIOMEDICAL AND LIFE SCIENCES

ETHICS COMMITTEE FOR NON CLINICAL RESEARCH INVOLVING HUMAN SUBJECTS, MATERIAL OR DATA

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

A submission to this Committee does not automatically result in approval. Investigators must wait for written approval before commencing data collection. Disciplinary measures will be taken if work commences without ethical approval being in place. The matter will be referred to the Dean for appropriate action.

THIS APPLICATION FORM SHOULD BE TYPED, NOT HAND WRITTEN.

ALL QUESTIONS MUST BE ANSWERED. "NOT APPLICABLE" IS A SATISFACTORY ANSWER WHERE APPROPRIATE.

Project Title: **The effects of glycine-arginine-[alpha]-ketoisocaproic acid on fatigue during repeated cycle sprints in trained humans.**

Is this project from a commercial source? **Yes**

If yes, give details and ensure that this is stated on the Informed Consent form.

This project is supported by Iovate Health Sciences Research Inc, Canada.

Date of submission: **March 2006**

Name of all person(s) submitting research proposal: **Dr Yannis Pitsiladis**

Position(s) held: **Reader in Exercise Physiology**

Division: **NABS**

Address for correspondence relating to this submission: **Lab 245, West Medical Building. Phone: 0141 330 3858, email: Y.Pitsiladis@bio.gla.ac.uk**

Name of Principal Researcher (if different from above e.g., Student's Supervisor)

Position held

1. Describe the purposes of the research proposed.

Dynamic exercise performance can be limited by a reduction in muscular power output during high intensity, exhaustive exercise (Lewis & Fulco, 1998). Numerous dietary supplements that potentially limit the reduction in muscle power output during exercise have been examined to date, with equivocal results (Williams, 1999). Lack of understanding as to the exact mechanism of fatigue and difficulties in reproducibility and objectively measuring high intensity performance are likely to account for these differences.

In vitro experiments and clinical studies have demonstrated that metabolic manipulation of nitrogen metabolism using selected ketoacids alone, or in conjunction with certain amino acids, can reduce various pathophysiologoical effects of trauma, including clinically dysfunctional skeletal muscle (e.g Hirokawa & Walser, 1999; Sandstedt et al. 1992). Stevens et al. (2000) have determined that ingestion of a glycine and L-arginine salt of [alpha]-ketoisocaproic acid calcium ("GAKIC") significantly increased resistance to fatigue by up to 28% and on average increased total muscle work by approximately 11% measured on a computer-controlled isokinetic dynamometer. Furthermore, Buford & Koch (2004) have recently demonstrated that GAKIC is associated with a greater retention of mean power measured between the 1st and 2nd sprints during five consecutive sprints on a cycle ergometer. Despite these measured ergogenic effects of GAKIC on resistance to skeletal muscle fatigue, the exact mechanism through which GAKIC may have imparted these performance gains remains to be determined. Of further potential interest, is the apparent increased peak power produced after GAKIC supplementation during the 5th and final sprint. If GAKIC can increase fatigue resistance then it would be expected that an increased cycle-sprint power output would be maintained after 5 sets of sprints. Therefore the aim of the proposed investigation is determine the effects of GAKIC supplementation on skeletal muscle power and fatigue measured during 10 consecutive 10 s cycle sprints. The study will also provide further information into the potential mechanism underlying the ergogenic potential of GAKIC.

2. Please give a summary of the design and methodology of the project. Please also include in this section details of the proposed sample size, giving indications of the calculations used to determine the required sample size, including any assumptions you may have made (if in doubt, please obtain statistical advice).

Methods/Design of investigation

We propose to study 10 trained male subjects (17-35 yrs) (this sample size is in line with the statistical procedures to be used). Subjects will be in good health at the time of testing and regularly take part in strenuous exercise. Eligibility will be assessed by subjects undergoing a medical examination (as previously approved by the University Ethics Committee). Subjects will also be required to read and sign the enclosed information sheet.

Protocols

Subjects will visit the laboratory on three separate occasions. The first visit will be used to explain experimental procedures, collect anthropometric data and record baseline power data. On the second and third session subjects will receive experimental treatments (GAKIC or Pl) and will be randomly assigned in a double blind, crossover fashion separated by 7 days. Subjects will be required to complete a 24-h dietary record the day prior to each test, which will subsequently be analysed for EI and amino acids.

Experimental treatments

Subjects will arrive in the laboratory after an overnight fast before consuming the treatment beverage (GAKIC or Pl) in three equal aliquots over a 45 min period. The GAKIC supplement will consist of glycine-L-arginine-[alpha]-ketoisocaproic acid (2.0 g glycine plus 6.0 g L-arginine monohydrochloride plus 3.2 g [alpha]-ketoisocaproic acid calcium salt) (GAKIC, Iovate Health Sciences International Inc, Mississauga, Canada). Based on the 2002/2005 DRI report, the recommended dietary allowance of protein and amino acids in the diet is 56 g per day for males aged 19-30 years. The isocaloric Pl will be composed of 9.46 g sucrose. The GAKIC or Pl will be dissolved in chilled cranberry juice, divided into three equal portions and consumed by the subject at 45, 30 and 10 min before exercise.

Exercise trials

After warm-up subjects will perform a series of ten, 10 s sprints on a Lode-Excalibur sport electronically brake cycle ergometer. Each sprint will be separated by a 50 s rest interval. Each sprint will be a modified 10 s Wingate test performed using a resistance of 0.8 $N \cdot kg^{-1}$. Each sprint will be initiated from a dead stop and analysed using computer software for power output at each second of the sprint allowing calculation of both 10 second mean power and peak power output. Furthermore, the fatigue index will be determined using the following equation: fatigue index % = [(peak power – minimum power)/peak power] x 100 where minimum power is the lowest power output after the subject achieved peak.

3. Describe the research procedures as they affect the research subject and any other parties involved.

All experiments will take place in the Laboratory of Human Physiology in the West Medical Building. Dr Yannis Pitsiladis or a qualified (CPR-trained) and experienced colleague will be present at all tests. Dr Pitsiladis is trained in CPR and Advanced Life Support.

Potential participants will be identified either by personal contact or by advertisement. They will be asked to meet with the investigators to discuss the project and whether they would be suitable as a subject. All subjects will be healthy individuals without a history of any significant medical problem(s). All subjects will be endurance-trained and therefore accustomed to strenuous exercise to exhaustion. The good health of each subject will be established prior to the study by subjects undergoing a medical examination (as previously approved by the University Ethics Committee), which is supported by a written assurance from the subject. Subjects with a history of cardiorespiratory or neurological disease will be excluded from participation, as will those having an acute upper respiratory tract infection. Subjects who take drugs (recreational or performance enhancing drugs) or who have consumed alcohol within 48 h of an experiment will be excluded.

Close supervision of the subject is ensured at all times by the supervising investigator. The well-being of the subject is established at frequent intervals throughout all tests by asking the subject "Is everything alright?" Subjects are instructed, prior to the test, to respond to this question with a thumbs-up sign if everything is fine, and a thumbs-down sign if there is problem. If a problem is indicated, the investigator will ask further

questions to establish whether there is a technical problem that could lead to potential hazard or whether the subject is feeling unwell. In either case, the test is immediately halted. All subjects are routinely instructed to cease exercising if they experience any discomfort or have any concern for their well-being.

The risks associated with performing maximal exercise are minimal as long as the subject is appropriately instructed and familiarised with the device prior to participation and also is appropriately supervised during the experiment. All exercise bouts are both preceded by a 5 min "warm-up" and by a 5 min "warm-down". The latter is of particular importance during high-intensity exercise, when the local accumulation of exercise metabolites can cause an "expansion" (or vasodilatation) of the blood vessels in the lower limbs, which can impair the adequate return of blood to the heart – predisposing to fainting on dismounting from the ergometer. This risk is minimised by having the subject exercise at a mild level during recovery to "wash away" these metabolites and therefore to restore the capacity of the involved blood vessels to their resting levels.

4. What in your opinion are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

Exercise has negligible risk in healthy adults, although maximal exercise has a small risk of inducing myocardial ischaemia.

The subjects will complete a medical questionnaire and provide their written consent with the option to withdraw from training or testing at any point.

There have been no reported side-effects to GAKIC ingestion using the same supplementation dose and frequency as the present study (e.g. Stevens et al. 2000; Buford & Koch (2004) and GAKIC is commercially available at select food supplement stores.

5. Outline the reasons which lead you to be satisfied that the possible benefits to be gained from the project justify any risks or discomforts involved.

It is envisaged that this research will benefit the identification of the physiological mechanisms which limit exercise tolerance (i.e., the ability of individuals to perform exercise) during high intensity dynamic exercise. The minimal risk and discomfort

associated with the above procedures are considered to be worthwhile to gain the information required.

6. Who are the investigators (including assistants) who will conduct the research and what are their qualifications and experience?

Dr Yannis Pitsiladis PhD MMedSci BA, Chris Easton BSc, Mr John Wilson, Mrs Heather Collin (Senior Technicians), and 3 BSc Honours Project Students. The principal investigators have wide ranging experience of exercise testing over periods of up to 10 years without incident. The principal researchers have carried out Cr supplementation studies and exercise to exhaustion studies in relatively extreme environmental conditions in the past.

7. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

In the event of an emergency, guidelines recently approved by the ethics committee will be followed.

In the event of an untoward incident that is not an emergency, the supervising Principal Investigator will administer appropriate first aid, if necessary. The subject will not be permitted to leave the laboratory until he has fully recovered. The subject will be encouraged to contact his local GP. The subject will be told that one of the Principal Investigators will conduct a follow-up by telephone at the end of the same day. The subject will also be provided with 24-h contact numbers for both Principal Investigators.

8. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital) describe the arrangements you intend to make to gain access to this information including, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

N/A

9. Specify whether subjects will include students or others in a dependent relationship.

Some students may be recruited but will be under no pressure from staff to participate in the study.

10. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects.

N/A

11. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering payment or other incentive.

No.

12. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Each subject will be provided with a consent form outlining the testing procedures, which asks them for their written consent to participate in the project with the option to withdraw at any time (see enclosed copy). A verbal explanation will also be given and any queries answered. If there is some doubt of the subject's eligibility for the study, the subject will be excluded. Information on GAKIC supplementation and the Pl will be given in the Information Sheet.

13. Comment on any cultural, social or gender-based characteristics of the subject which have affected the design of the project or which may affect its conduct.

All subjects are male. This constraint is imposed for standardisation purposes

14. Please state who will have access to the data and what measures which will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g., will the data be anonymised?

The information obtained will be anonymised and individual information will not be passed on to anyone outside the study group. The results of the tests will not be used for selection purposes.

15. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

No

16. Date on which the project will begin **April 2006** and end **April 2007**

17. Please state location(s) where the project will be carried out.

Laboratory of Human Physiology (Lab 245), West Medical Building.

18. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken

All experiments will be conducted according to standard health and safety procedures.

Signed _____ Date _____(Proposer of research)

Where the proposal is from a student, the Supervisor is asked to certify the accuracy of the above account.

References

1. Buford BN & Kock AJ (2004). Glycine-Arginine-[alpha]-ketoisocaproic acid improves performance of repeated cycling sprints. *Med Sci Sports Exerc*, 36: 583-587.

2. Hirokawa M & Walser M (1999). Enternal infusion of sodium 2-ketoisocaproate in endotoxic rats. *Crit Care Med*, 27: 373-379.

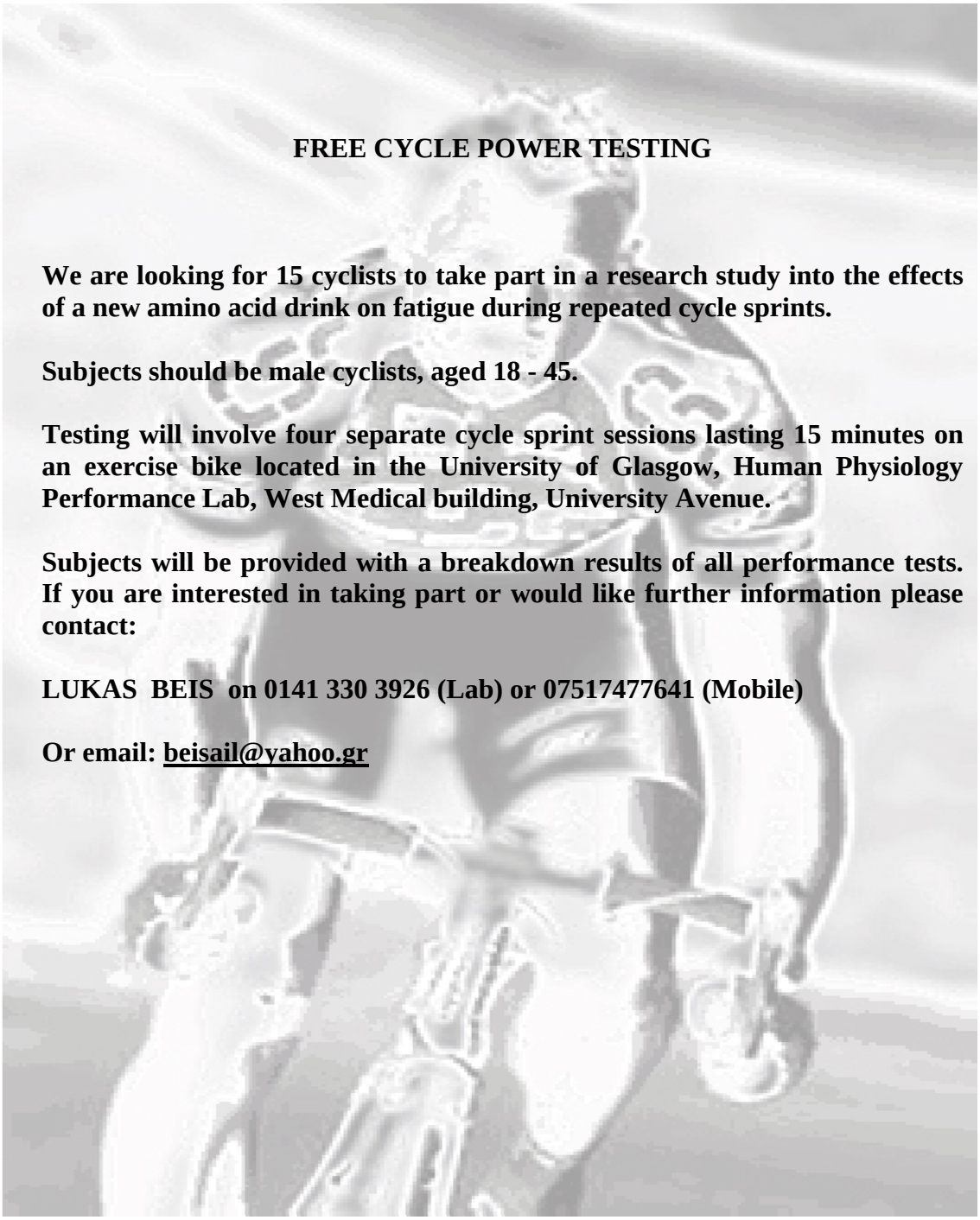
3. Lewis SF & Fulco CS (1998). A new approach to studying muscle fatigue and factors affecting performance during dynamic exercise in humans. *Exerc Sport Sci Rev*, 26: 91-116.

4. Sandstedt S, Jorfrldt L & Larsson J (1992). Randomized controlled study evaluating effects of branched chain amino acids and alpha-ketoisocaproate on protein metabolism after surgery. *Br J Surg*, 79: 217-220.

5. Stevens BR, Godfrey MD, Kaminski TW & Braith RW (2000). High-intensity dynamic human performance enhanced by a metabolic intervention. *Med Sci Sports Exerc*, 32: 2102-2108.

6. Williams MH (1999). Facts and fallacies of purported ergogenic amino acid supplements. *Clin Sports Med*, 18: 633-649.

Appendix D: Advertising leaflet



Appendix E: Ethics Document

UNIVERSITY OF GLASGOW
FACULTY OF BIOMEDICAL AND LIFE SCIENCES

ETHICS COMMITTEE FOR NON CLINICAL RESEARCH INVOLVING HUMAN SUBJECTS, MATERIAL OR DATA

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

A submission to this Committee does not automatically result in approval. Investigators must wait for written approval before commencing data collection. Disciplinary measures will be taken if work commences without ethical approval being in place. The matter will be referred to the Dean for appropriate action.

THIS APPLICATION FORM SHOULD BE TYPED, NOT HAND WRITTEN.

ALL QUESTIONS MUST BE ANSWERED. "NOT APPLICABLE" IS A SATISFACTORY ANSWER WHERE APPROPRIATE.

Project Title: **The effects of hyperhydration on running economy of endurance trained runners.**

Is this project from a commercial source? **No**

If yes, give details and ensure that this is stated on the Informed Consent form.

Date of submission: **October 2007**

Name of all person(s) submitting research proposal: **Dr Yannis Pitsiladis**

Position(s) held: Reader in Exercise Physiology

Division: **NABS**

Address for correspondence relating to this submission: **Lab 245, West Medical Building. Phone: 0141 330 3858, email: Y.Pitsiladis@bio.gla.ac.uk**

Name of Principal Researcher (if different from above e.g., Student's Supervisor): **N/A**

1. Describe the purposes of the research proposed.

The vast majority of the differences in endurance running performance in elite athletes are principally accounted for by running economy with maximal aerobic capacity ($\dot{V}O_{2max}$) and % $\dot{V}O_2$max also having a significant impact (Bassett & Howley, 2000; Conley & Krahenbuhl, 1980). Running economy is defined as the rate of oxygen utilization ($\dot{V}O_2$) required running at a sub-maximal given velocity and is expressed as the $\dot{V}O_2$ per unit

mass per min ($mL \cdot kg^{-1} \cdot min^{-1}$) (Bassett & Howley, 2000). Athletes who maintain a lower $\dot{V}O_2$ while running at a given velocity use less energy and therefore are said to have a better running economy. Subsequently, if BM can be reduced and power output maintained, athletes should theoretically use less energy and therefore more economical. This physiological phenomenon may help explain why despite the reported negative impact of dehydration on thermoregulatory and cardiovascular parameters (Montain & Coyle, 1992) athletes consistently perform well during competition despite losing between 4 and 8% BM (Coyle, 2004). Conversely, hyper-hydrating prior to competing in weight bearing sports such as running via ingestion of osmotic agents such as Gly or Cr (Easton et al., 2007) may negatively impact performance due to the associated increase in BM (Noakes, 2003a).

However, the only published study to date to examine the effects of hydration status on running economy found dehydration of up to 5% BM had no effect on running economy and resulted in a significant increase in physiological strain during running in 23 ^{o}C (Armstrong et al., 2006). Nevertheless, it is currently unknown whether hyper-hydrating, and thus increasing BM would negatively impact on running economy. Therefore, the primary aim of the present study is to examine the effects of combined Cr and Gly hyper-hydration on running economy, cardiovascular, thermoregulatory and metabolic responses to running exercise in both cold (10 ^{o}C) and hot (35 ^{o}C) conditions in endurance trained subjects. A secondary aim of this study is to determine the accuracy of a breath-by-breath portable gas analyser, (K4, COSMED s.r.l., Rome, Italy) for measurement of gas exchange variables and running economy in the field.

2. Please give a summary of the design and methodology of the project. Please also include in this section details of the proposed sample size, giving indications of the calculations used to determine the required sample size, including any assumptions you may have made. (If in doubt, please obtain statistical advice).

Methods/Design of investigation

We propose to study 12 endurance-trained males (mostly runners aged 17-40 yrs). Subjects will be in good health at the time of testing and regularly take part in strenuous exercise. Eligibility will be assessed by subjects undergoing a medical examination. Subjects will also be required to read and sign the enclosed information sheet and a high intensity consent form (as used in studies previously accepted by the FBLS ethics

committee, e.g., The effects of combined Cr and Gly supplementation on hydration, thermoregulation and exercise performance in the heat in endurance-trained subjects).

Testing will take place in the laboratory of human physiology in the West Medical Building. A series of assessments will be carried out. These will include (see protocols): body composition using standard anthropometric methods; extracellular water and total body water measurement using multi-frequency bioelectrical impedance and 6 exercise tests. During the first laboratory visit, a health questionnaire and physical examination will be completed, the study and equipment will be explained to the subjects, and the subjects $\dot{V}O_2$max will be measured. The subsequent 5 tests will involve 4 visits to the laboratory and 1 to the running track. Following measurement of $\dot{V}O_2$max, two familiarisation and 1 baseline exercise trials, subjects will be ingest Cr and Gly for a period of 7 days.

Each supplement will consist of 11.4 g of Cr H2O (equivalent to 10 g Cr), 75 g of glucose polymer and 1g/kg BM Gly made up in 1 L of warm to hot water (x 2 times daily) and flavoured with sugar free fruit juice as necessary. Subjects will ingest the final Cr/glucose/Gly drink 5 h before performing a post-supplementation exercise trial and further 500 mL water 1 h before the test. Subjects will be instructed to carry out a weighed intake of food and an activity diary in the 24-h preceding each test. This supplementation regiment has previously been approved by the university ethics committee and as anticipated, no subjects experienced side-effects.

Protocols

Tests 1 and 2: Discontinuous Incremental Maximal Speed Test: will take place both in the laboratory and in the field. Subjects will be instructed to run at 10, 11, 12, 13, 14, 15, 16, 17 and 18 $km \cdot h^{-1}$ (or until volitional exhaustion) for 3 min at each speed, with a 3-5 min rest interval between each bout walking at 4 $km \cdot h^{-1}$. The laboratory test will be performed on a motorised treadmill, during which, the gradient shall remain at 1% throughout. Whereas the field test will take place on a 400m running track; an investigator will cycle alongside the subject to control the speed using a GPS system (GPSports SPI 10, GPSSports, Fyshwick ACT, Australia).

Test 3, 4, 5 and 6: Running Economy Tests: will take place in the laboratory on a motorised treadmill. Subjects will be required to run at a constant pace of 60% $\dot{V}O_2$max for 30 min in 10 ^{o}C and 70% relative humidity. Following this, subjects will receive a 35 min

rest period during which time any fluid lost during exercise will be replaced via ingestion of water. Subjects will then be required to run for a further 30 min at a constant pace of 60% $\dot{V}O_2$max for 30 min in 35 ^{o}C and 70% relative humidity.

Subjects will be given a warm-up before, and a warm-down after all four tests. The subject will wear a triaxial accelerometer (3dNX, Biotel, Bristol, U.K.) to record the activity counts per min. HR (Suunto t6, Suunto Oy, Vantaa, Finland) and gas exchange variables (Douglas bag collection) will be measured throughout exercise. Each subject will be required to swallow a CorTemp Ingestible Temperature Sensor the evening prior to each test. The pill is a small electronic device, which measures T_{core} and transmits it through a radio wave signal to an external receiver (Rav-Acha et al, 2003).

Methods/Design of investigation (continued)

Expired gas values obtained from Douglas bag analysis in experiments 1 and 2 will be compared to data previously collected using a K4 metabolic analyser using similar experimental protocols. In exercise tests 3, 4, 5 and 6 a venous cannula will be inserted and a 10 mL blood sample taken before and after each bout of exercise

Bioelectrical impedance: Extracellular water and total body water will be prior to exercise tests 3, 4 and 5 using multi-frequency bioelectrical impedance (Bodystat Multiscan 500). This non-invasive method involves placing two current-inducing electrodes and two detector electrodes on the dorsal surfaces of the right hand and foot and a small (and imperceptible) electrical current (500 Micro-Amps) introduced between these.

3. Describe the research procedures as they affect the research subject and any other parties involved.

All experiments will take place in the Environmental Chamber in the West Medical Building. Dr Yannis Pitsiladis or a qualified (CPR-trained) and experienced colleague will be present at all tests. Dr Pitsiladis is a certified phlebotomist and trained in CPR and Advanced Life Support.

Some subjects may experience mild discomfort during the placement of and/or sampling of blood from a catheter placed in a vein on the dorsum of the heated hand. In our experience, this is minimal because: the catheter size is small (20 G); it is only placed

when the hand has been heated to 44 °C for at least 10 min, allowing a substantial local vasodilatation of the superficial blood vessels, which facilitates their cannulation; the catheter is indwelling, allowing for multiple sampling; while it is safely secured in place with adhesive tape, there is sufficient 'play' to allow sampling without 'pulling' on the vessel (i.e., the catheter can slide easily within the vessel); upon withdrawal of the catheter at the end of the experiment, firm pressure is maintained over the site to prevent any leakage from the vessel into the surrounding interstitium which could lead to local oedema and bruising. Importantly, if a vessel cannot be readily cannulated or if the subject is nonetheless not comfortable with proceeding, the experiment is halted. No more than 30 mL of blood will be sampled for each test.

Potential participants will be identified either by personal contact or by advertisement. They will be asked to meet with the investigators to discuss the project and whether they would be suitable as a subject. All subjects will be endurance trained individuals without a history of any significant medical problem(s). The good health of each subject will be established prior to the study by subjects undergoing a medical examination by a qualified medical practitioner (as previously approved by the University Ethics Committee), which is supported by written assurance from the subject in the form of a detailed medical questionnaire. Subjects with a history of cardiorespiratory or neurological disease will be excluded from participation, as will those having an acute upper respiratory tract infection. Subjects who take drugs (recreational or performance enhancing drugs) or who have consumed alcohol within 48 h of an experiment will be excluded.

Exercise testing: The risks associated with performing maximal exercise are minimal as long as the subject is appropriately instructed and familiarised with the device prior to participation and also is appropriately supervised during the experiment. All exercise bouts are both preceded by a 5 min "warm-up" and by a 5 min "warm-down". The latter is of particular importance during high-intensity exercise, when the local accumulation of exercise metabolites can cause an "expansion" (or vasodilatation) of the blood vessels in the lower limbs, which can impair the adequate return of blood to the heart – predisposing to fainting on dismounting from the ergometer. This risk is minimised by having the subject exercise at a mild level during recovery to "wash away" these metabolites and therefore to restore the capacity of the involved blood vessels to their resting levels.

Some subjects experience difficulty swallowing while breathing through a mouthpiece and wearing a nose clip, due to some transient build-up of pressure in the ears.

4. What in your opinion are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

All procedures described in this application have previously been approved by the University Ethics Committee and carried out without incident (e.g. The effects of combined Cr and Gly supplementation on hydration, thermoregulation and exercise performance in the heat in endurance-trained subjects; The effects of combined Cr and Gly hyper-hydration on cardiovascular responses to postural change; Estimation of aerobic power from accelerometers and HR during walking and running.).

The subjects will complete a medical questionnaire and provide their written consent with the option to withdraw from participation at any point.

The insertion of a catheter into a vein may rarely cause irritation at the site of insertion, venospasm (or constriction of the cannulated vein which may lead to interference with blood flow through it) and phlebitis. These risks are minimized in this investigation by the short duration of the test and by the procedures described above.

Blood will be handled, stored and disposed of according to standard health and safety procedures.

Possible side-effects from the use of similar Gly hyper-hydration strategies include slight nausea, gastrointestinal distress (diarrhoea) and headaches. These problems have been reported among some subjects in the many published studies to date (Easton et al., 2007; Freund et al., 1995; Lyons et al., 1990).

The only known 'side effect' of oral Cr supplementation that has been reported is a temporary increase in body weight.

5. Outline the reasons which lead you to be satisfied that the possible benefits to be gained from the project justify any risks or discomforts involved.

It is envisaged that this research will identify whether an increase in BM induced by hyper-hydration will negatively affect running economy. The minimal risk and discomfort associated with the above procedures are considered to be worthwhile to gain the information required.

6. Who are the investigators (including assistants) who will conduct the research and what are their qualifications and experience?

Dr Yannis Pitsiladis PhD MMedSci BA, Dr Chris Easton BSc PhD, Mr John Wilson, (Senior Technician), and BSc Honours Project and MRes Students. The principal investigators have wide ranging experience of physiological testing over periods of up to 10 years without incident. The principal researchers have carried out Cr/Gly supplementation studies and exercise to exhaustion studies in relatively extreme environmental conditions in the past.

7. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

In the event of an emergency, guidelines recently approved by the ethics committee will be followed.

In the event of an untoward incident that is not an emergency, the supervising Principal Investigator will administer appropriate first aid, if necessary. The subject will not be permitted to leave the laboratory until he has fully recovered. The subject will be encouraged to contact his local GP. The subject will be told that one of the Principal Investigators will conduct a follow-up by telephone at the end of the same day. The subject will also be provided with 24-h contact numbers for both Principal Investigators.

8. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital) describe the arrangements you intend to make to gain access to this information including, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

N/A

9. Specify whether subjects will include students or others in a dependent relationship.

Some students may be recruited but will be under no pressure from staff to participate in the study. Steps will be taken to avoid the recruitment of students in a dependent relationship with the academic involved in the study.

10. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects.

N/A

11. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering payment or other incentive.

No

12. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Each subject will be provided with a consent form outlining the testing procedures, which asks them for their written consent to participate in the project with the option to withdraw at any time (see enclosed copy). A verbal explanation will also be given and any queries answered. If there is some doubt of the subject's eligibility for the study, the subject will be excluded. Information on Cr and Gly supplementation will be given in the Information Sheet.

13. Comment on any cultural, social or gender-based characteristics of the subject which have affected the design of the project or which may affect its conduct.

All subjects are male. This constraint is imposed for standardisation purposes.

14. Please state who will have access to the data and what measures which will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g., will the data be anonymised?

The information obtained will be anonymised and individual information will not be passed on to anyone outside the study group. The results of the tests will not be used for selection purposes.

15. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

No

16. Date on which the project will begin **November 2007** and end **May 2008**

17. Please state location(s) where the project will be carried out.

Laboratory of Human Physiology, West Medical Building.

Outdoor track (Scotstoun stadium and Bellahouston Park).

18. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g., where blood samples are being taken.

All experiments will be conducted according to the Code of Practice for conducting experiments in non-patient human volunteers (including handling and disposal of human blood, urine and sputum) previously accepted by the University Ethics Committee.

Reference List

1. Armstrong, L. E., Whittlesey, M. J., Casa, D. J., Elliott, T. A., Kavouras, S. A., Keith, N. R., & Maresh, C. M. (2006). No effect of 5% hypohydration on running economy of competitive runners at 23 degrees C. Medicine and Science in Sports and Exercise 38, 1762-1769.

2. Bassett, D. R., Jr. & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med.Sci.Sports Exerc. 32, 70-84.

3. Conley, D. L. & Krahenbuhl, G. S. (1980). Running economy and distance running performance of highly trained athletes. Med.Sci.Sports Exerc. 12, 357-360.

4. Coyle, E. F. (2004). Fluid and fuel intake during exercise. Journal of Sports Sciences 22, 39-55.

5. Easton, C., Turner, S., & Pitsiladis, Y. P. (2007). Cr and Gly hyperhydration in trained subjects before exercise in the heat. International Journal of Sport Nutrition and Exercise Metabolism 17, 70-91.

6. Freund, B. J., Montain, S. J., Young, A. J., Sawka, M. N., Deluca, J. P., Pandolf, K. B., & Valeri, C. R. (1995). Glycerol hyperhydration: Hormonal, renal, and vascular fluid responses. Journal of Applied Physiology 79, 2069-2077.

7. Lyons, T. P., Riedesel, M. L., Meuli, L. E., & Chick, T. W. (1990). Effects of Glycerol-Induced Hyperhydration Prior to Exercise in the Heat on Sweating and Core Temperature. Medicine and Science in Sports and Exercise 22, 477-483.

8. Montain, S. J. & Coyle, E. F. (1992). Influence of Graded Dehydration on Hyperthermia and Cardiovascular Drift During Exercise. Journal of Applied Physiology 73, 1340-1350.

9. Noakes, T. D. (2003). Ergogenic Aids. In The lore of running, ed. Noakes, T. D., Human Kinetics, Champaign.

Appendix F: Health Screening and Physical Activity Questionnaire

UNIVERSITY OF GLASGOW
INSTITUTE OF BIOMEDICAL AND LIFE SCIENCES

SUBJECT'S QUESTIONNAIRE AND ASSENT FORM FOR HIGH-INTENSITY EXERCISE TESTING

If you feel unwell on the day of a proposed test, or have been feeling poorly over the preceding day or two, DO NOT TAKE PART in a high-intensity exercise test.
The considerations which follow apply to people who are feeling well at the time.

Name:

Sex (M/F) _____ Age _____ (yrs) Height _____ (m) Weight _____ (kg)

Exercise Lifestyle

a) What kind(s) of exercise do you regularly do (20 + min/session)? (*Please circle*)

	Number of times per average week				
Walking	1	2	3	4	5
Running	1	2	3	4	5
Cycling	1	2	3	4	5
Swimming	1	2	3	4	5
Skiing	1	2	3	4	5
Rowing	1	2	3	4	5
Gymnastics	1	2	3	4	5
Martial arts	1	2	3	4	5
Tune Up	1	2	3	4	5
Popmobility	1	2	3	4	5
Sweat Session	1	2	3	4	5
Field athletics	1	2	3	4	5
Weight training	1	2	3	4	5
Racquet sports	1	2	3	4	5
Rugby/soccer/hockey	1	2	3	4	5
Other(s) *	1	2	3	4	5

* (*Please specify)* _____

b) How long have you been exercising at least twice/week for at least 20 min/session?

_____

Continued Over

Smoking
(*Please tick one)*

Never smoked	_____
Not for > 6 months	_____
Smoke <10 per day	_____
Smoke > 10 per day	_____

Illnesses
Have you ever had …? (*Please circle Yes or No*)

Asthma	YES	NO
Diabetes	YES	NO
Epilepsy	YES	NO
Heart Disease	YES	NO
High Blood Pressure	YES	NO

Any other illness that could affect your safety in performing maximal exercise

YES NO

(If YES, please specify) _____

Symptoms
Have you ever had any of the following symptoms to a significant degree?
i.e., have you had to consult a physician relating to any of the following?
(*Please circle Yes or No*)

Breathlessness	YES	NO
Chest Pain	YES	NO
Dizzy fits / Fainting	YES	NO
Heart Murmurs	YES	NO
Palpitations	YES	NO

Muscle or joint injury

Do you have / or have had any muscle or joint injury which could affect your safety in performing maximal exercise or strength testing or strength training?

YES NO

Medication
Are you currently taking any medication? YES NO
(*Please circle Yes or No*)

(*If Yes, please specify*) _____

Signature _____

Date _____

Signed _____ Date _____ (Proposer of research)

Where the proposal is from a student, the Supervisor is asked to certify the accuracy of the above account.

Signed _____Date _____(Supervisor of student)

Email the completed form to: S.Morrison@bio.gla.ac.uk

And send the signed hard copy to:

Stuart Morrison
Faculty Research Office
Faculty of Biomedical & Life Sciences
West Medical Building
University of Glasgow
Gilmorehill
Glasgow
G12 8QQ

List of References

1. Abdel-Rahman, A., Anyangwe, N., Carlacci, L., Casper, S., Danam, R. P., Enongene, E., Erives, G., Fabricant, D., Gudi, R., Hilmas, C. J., Hines, F., Howard, P., Levy, D., Lin, Y., Moore, R. J., Pfeiler, E., Thurmond, T. S., Turujman, S., & Walker, N. J. (2011). The safety and regulation of natural products used as foods and food ingredients. *Toxicol Sci, 123*(2), 333-348

2. ACSM. (2000). Position of Dietitians of Canada, the American Dietetic Association, and the American College of Sports Medicine: Nutrition and Athletic Performance. *Can J Diet Pract Res, 61*(4), 176-192

3. Ahrendt, D. M. (2001). Ergogenic aids: counseling the athlete. *Am Fam Physician, 63*(5), 913-922

4. Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., Swartz, A. M., Strath, S. J., O'Brien, W. L., Bassett, D. R., Jr., Schmitz, K. H., Emplaincourt, P. O., Jacobs, D. R., Jr., & Leon, A. S. (2000). Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc, 32*(9 Suppl), S498-504

5. Almond, C. S., Shin, A. Y., Fortescue, E. B., Mannix, R. C., Wypij, D., Binstadt, B. A., Duncan, C. N., Olson, D. P., Salerno, A. E., Newburger, J. W., & Greenes, D. S. (2005). Hyponatremia among runners in the Boston Marathon. *N Engl J Med, 352*(15), 1550-1556

6. Alves, C., & Lima, R. V. (2009). Dietary supplement use by adolescents. *J Pediatr (Rio J), 85*(4), 287-294

7. Anderson, M. J., Cotter, J. D., Garnham, A. P., Casley, D. J., & Febbraio, M. A. (2001). Effect of glycerol-induced hyperhydration on thermoregulation and metabolism during exercise in heat. *Int J Sport Nutr Exerc Metab, 11*(3), 315-333

8. Armstrong, L. E., Costill, D. L., & Fink, W. J. (1985). Influence of diuretic-induced dehydration on competitive running performance. *Med Sci Sports Exerc, 17*(4), 456-461

9. Armstrong, L. E., Maresh, C. M., Gabaree, C. V., Hoffman, J. R., Kavouras, S. A., Kenefick, R. W., Castellani, J. W., & Ahlquist, L. E. (1997). Thermal and circulatory responses during exercise: effects of hypohydration, dehydration, and water intake. *J Appl Physiol, 82*(6), 2028-2035

10. Armstrong, L. E., Whittlesey, M. J., Casa, D. J., Elliott, T. A., Kavouras, S. A., Keith, N. R., & Maresh, C. M. (2006). No effect of 5% hypohydration on running economy of competitive runners at 23 degrees C. *Med Sci Sports Exerc, 38*(10), 1762-1769

11. Bassett, D. R., Jr., & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc, 32*(1), 70-84

12. Besset, A., Bonardet, A., Rondouin, G., Descomps, B., & Passouant, P. (1982). Increase in sleep related GH and Prl secretion after chronic arginine aspartate administration in man. *Acta Endocrinol (Copenh), 99*(1), 18-23

13. Binkley, H. M., Beckett, J., Casa, D. J., Kleiner, D. M., & Plummer, P. E. (2002). National Athletic Trainers' Association Position Statement: Exertional Heat Illnesses. *J Athl Train, 37*(3), 329-343

14. Blomstrand, E., Hassmen, P., Ekblom, B., & Newsholme, E. A. (1991). Administration of branched-chain amino acids during sustained exercise--effects on performance and on plasma concentration of some amino acids. *Eur J Appl Physiol Occup Physiol, 63*(2), 83-88

15. Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc, 14*(5), 377-381

16. Bower, R. H., Cerra, F. B., Bershadsky, B., Licari, J. J., Hoyt, D. B., Jensen, G. L., Van Buren, C. T., Rothkopf, M. M., Daly, J. M., & Adelsberg, B. R. (1995). Early enteral administration of a formula (Impact) supplemented with arginine, nucleotides, and fish oil in intensive care unit patients: results of a multicenter, prospective, randomized, clinical trial. *Crit Care Med, 23*(3), 436-449

17. Buford, B. N., & Koch, A. J. (2004). Glycine-arginine-alpha-ketoisocaproic acid improves performance of repeated cycling sprints. *Med Sci Sports Exerc, 36*(4), 583-587

18. Burke, L. M., Castell, L. M., & Stear, S. J. (2009). BJSM reviews: A-Z of supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance Part 1. *Br J Sports Med, 43*(10), 728-729

19. Burke, L. M., Gollan, R. A., & Read, R. S. (1991). Dietary intakes and food use of groups of elite Australian male athletes. *Int J Sport Nutr, 1*(4), 378-394

20. Calfee, R., & Fadale, P. (2006). Popular ergogenic drugs and supplements in young athletes. *Pediatrics, 117*(3), e577-589

21. Campbell, B. I., La Bounty, P. M., & Roberts, M. (2004). The ergogenic potential of arginine. *J Int Soc Sports Nutr, 1*(2), 35-38

22. Casa, D. J., Armstrong, L. E., Hillman, S. K., Montain, S. J., Reiff, R. V., Rich, B. S., Roberts, W. O., & Stone, J. A. (2000). National Athletic Trainers' Association Position Statement: Fluid Replacement for Athletes. *J Athl Train, 35*(2), 212-224

23. Casa, D. J., Clarkson, P. M., & Roberts, W. O. (2005). American College of Sports Medicine roundtable on hydration and physical activity: consensus statements. *Curr Sports Med Rep, 4*(3), 115-127

24. Cerqueira, M. T., Fry, M. M., & Connor, W. E. (1979). The food and nutrient intakes of the Tarahumara Indians of Mexico. *Am J Clin Nutr, 32*(4), 905-915

25. Cheuvront, S. N., Carter, R., 3rd, Montain, S. J., Stephenson, L. A., & Sawka, M. N. (2004). Influence of hydration and airflow on thermoregulatory control in the heat. *Journal of Thermal Biology, 29*, 471-477

26. Cheuvront, S. N., Carter, R., 3rd, & Sawka, M. N. (2003). Fluid balance and endurance exercise performance. *Curr Sports Med Rep, 2*(4), 202-208

27. Cheuvront, S. N., & Haymes, E. M. (2001a). Ad libitum fluid intakes and thermoregulatory responses of female distance runners in three environments. *J Sports Sci, 19*(11), 845-854

28. Cheuvront, S. N., & Haymes, E. M. (2001b). Thermoregulation and marathon running: biological and environmental influences. *Sports Med, 31*(10), 743-762

29. Christensen, D. L., Van Hall, G., & Hambraeus, L. (2002). Food and macronutrient intake of male adolescent Kalenjin runners in Kenya. *Br J Nutr, 88*(6), 711-717

30. Coleman, D. A., Wiles, J. D., Nunn, M., & Smith, M. F. (2005). Reliability of sprint test indices in well-trained cyclists. *Int J Sports Med, 26*(5), 383-387

31. Conley, D.L., Krahenbuhl, G.S., Burkett, L.N., & Millar, A.L. (1984). Following Steve Scott: physiological changes accompanying training. *Phys Sports med, 12*, 103-106

32. Convertino, V. A., Armstrong, L. E., Coyle, E. F., Mack, G. W., Sawka, M. N., Senay, L. C., Jr., & Sherman, W. M. (1996). American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc, 28*(1), i-vii

33. Costill, D. L. (1972). Physiology of marathon running. *JAMA, 221*(9), 1024-1029

34. Costill, D. L., Kammer, W. F., & Fisher, A. (1970). Fluid ingestion during distance running. *Arch Environ Health, 21*(4), 520-525

35. Coyle, E. F. (2004). Fluid and fuel intake during exercise. *J Sports Sci, 22*(1), 39-55

36. Coyle, E. F. (2005). Improved muscular efficiency displayed as Tour de France champion matures. *J Appl Physiol, 98*(6), 2191-2196

37. Coyle, E. F., Feltner, M. E., Kautz, S. A., Hamilton, M. T., Montain, S. J., Baylor, A. M., Abraham, L. D., & Petrek, G. W. (1991). Physiological and biomechanical factors associated with elite endurance cycling performance. *Med Sci Sports Exerc, 23*(1), 93-107

38. Coyle, E. F., Jeukendrup, A. E., Oseto, M. C., Hodgkinson, B. J., & Zderic, T. W. (2001). Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise. *Am J Physiol Endocrinol Metab, 280*(3), E391-398

39. Crowe, M. J., Weatherson, J. N., & Bowden, B. F. (2006). Effects of dietary leucine supplementation on exercise performance. *Eur J Appl Physiol, 97*(6), 664-672

40. Cureton, K. J., & Sparling, P. B. (1980). Distance running performance and metabolic responses to running in men and women with excess weight experimentally equated. *Med Sci Sports Exerc, 12*(4), 288-294

41. Cureton, K. J., Sparling, P. B., Evans, B. W., Johnson, S. M., Kong, U. D., & Purvis, J. W. (1978). Effect of experimental alterations in excess weight on aerobic capacity and distance running performance. *Med Sci Sports, 10*(3), 194-199

42. Daly, J. M., Reynolds, J., Thom, A., Kinsley, L., Dietrick-Gallagher, M., Shou, J., & Ruggieri, B. (1988). Immune and metabolic effects of arginine in the surgical patient. *Ann Surg, 208*(4), 512-523

43. Daniels, J. T. (1985). A physiologist's view of running economy. *Med Sci Sports Exerc, 17*(3), 332-338

44. Daries, H. N., Noakes, T. D., & Dennis, S. C. (2000). Effect of fluid intake volume on 2-h running performances in a 25 degrees C environment. *Med Sci Sports Exerc, 32*(10), 1783-1789

45. Di Prampero, P. E., Capelli, C., Pagliaro, P., Antonutto, G., Girardis, M., Zamparo, P., & Soule, R. G. (1993). Energetics of best performances in middle-distance running. *J Appl Physiol, 74*(5), 2318-2324

46. Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol, 37*(2), 247-248

47. Douglas, C. G. (1911). A method for determining the total respiratory exchange in man. *J. Physiol. 42*, 17-18

48. Dugas, J. P., Oosthuizen, U., Tucker, R., & Noakes, T. D. (2009). Rates of fluid ingestion alter pacing but not thermoregulatory responses during prolonged exercise in hot and humid conditions with appropriate convective cooling. *Eur J Appl Physiol, 105*(1), 69-80

49. Easton, C., Fudge, B. W., & Pitsiladis, Y. P. (2007). Rectal, telemetry pill and tympanic membrane thermometry during exercise heat stress. *Journal of Thermal Biology*, *32*, 78-86.

50. Easton, C., Turner, S., & Pitsiladis, Y. P. (2007). Creatine and glycerol hyperhydration in trained subjects before exercise in the heat. *Int J Sport Nutr Exerc Metab, 17*(1), 70-91

51. Ekelund, L. G. (1967). Circulatory and respiratory adaptation during prolonged exercise. *Acta Physiol Scand Suppl, 292*, 1-38

52. Ethiopian Health and Nutrition Research Institute: Food Composition Table For Use In Ethiopia Part IV; 1998.

53. FDA (2011). USA: U.S. Food and Drug Administration: Retrieved Oct 12, 2011 from http://www.cfsan.fda.gov.

54. Fjeld, C. R., Brown, K. H., & Schoeller, D. A. (1988). Validation of the deuterium oxide method for measuring average daily milk intake in infants. *Am J Clin Nutr, 48*(3), 671-679

55. Fortney, S. M., Vroman, N. B., Beckett, W. S., Permutt, S., & LaFrance, N. D. (1988). Effect of exercise hemoconcentration and hyperosmolality on exercise responses. *J Appl Physiol, 65*(2), 519-524

56. Friedman, J. E., & Lemon, P. W. (1989). Effect of chronic endurance exercise on retention of dietary protein. *Int J Sports Med, 10*(2), 118-123

57. Fudge, B. W., Easton, C., Kingsmore, D., Kiplamai, F. K., Onywera, V. O., Westerterp, K. R., Kayser, B., Noakes, T. D., & Pitsiladis, Y. P. (2008). Elite Kenyan

endurance runners are hydrated day-to-day with ad libitum fluid intake. *Med Sci Sports Exerc, 40*(6), 1171-1179

58. Fudge, B. W., Pitsiladis, Y. P., Kingsmore, D., Noakes, T. D., & Kayser, B. Outstanding performances despite low fluid intakes: The Kenyan running experience. In: East African Running: Towards a Cross-Disciplinary Perspective. Pitsiladis YP, Bale J, Sharp C, Noakes TD (Eds.) United Kingdom: Routledge., 63-84; 2006a

59. Fudge, B. W., Westerterp, K. R., Kiplamai, F. K., Onywera, V. O., Boit, M. K., Kayser, B., & Pitsiladis, Y. P. (2006b). Evidence of negative energy balance using doubly labelled water in elite Kenyan endurance runners prior to competition. *Br J Nutr, 95*(1), 59-66

60. Fudge, B. W., Wilson, J., Easton, C., Irwin, L., Clark, J., Haddow, O., Kayser, B., & Pitsiladis, Y. P. (2007). Estimation of oxygen uptake during fast running using accelerometry and heart rate. *Med Sci Sports Exerc, 39*(1), 192-198

61. Gaine, P. C., Pikosky, M. A., Martin, W. F., Bolster, D. R., Maresh, C. M., & Rodriguez, N. R. (2006). Level of dietary protein impacts whole body protein turnover in trained males at rest. *Metabolism, 55*(4), 501-507

62. GAKIC (2010). Canada: GAKIC: Retrieved Jan 31, 2010 from: http://www.gakic.com.

63. Galloway, S. D., & Maughan, R. J. (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc, 29*(9), 1240-1249

64. Genton. (2011). Clinical Nutrition University: Calorie and macronutrient requirements for physical fitness.

65. Gisolfi, C. V., & Copping, J. R. (1974). Thermal effects of prolonged treadmill exercise in the heat. *Med Sci Sports, 6*(2), 108-113

66. Gisolfi, C. V., Summers, R. W., Schedl, H. P., Bleiler, T. L., & Oppliger, R. A. (1990). Human intestinal water absorption: direct vs. indirect measurements. *Am J Physiol, 258*(2 Pt 1), G216-222

67. Giunta, R., Basile, G., & Tibuzzi, A. (2010). Legislation on Nutraceuticals and Food Supplements: A Comparison between Regulations in USA and EU. *Adv Exp Med Biol, 698*, 322-328

68. Godek, S. F., Bartolozzi, A. R., & Godek, J. J. (2005). Sweat rate and fluid turnover in American football players compared with runners in a hot and humid environment. *Br J Sports Med, 39*(4), 205-211; discussion 205-211

69. Gonzalez-Alonso, J., Mora-Rodriguez, R., Below, P. R., & Coyle, E. F. (1995). Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol, 79*(5), 1487-1496

70. Gonzalez, R. R., Cheuvront, S. N., Montain, S. J., Goodman D. A., Blanchard L. A., Berglund L. G., & Sawka M. N. (2009). Expanded prediction equations of human sweat loss and water needs. *J Appl Physiol*, 107, 379-88.

71. Goulet, E. D. (2011). Effect of exercise-induced dehydration on time-trial exercise performance: a meta-analysis. *Br J Sports Med* (45), 1149-1156

72. Grandjean, A. C. (1989). Macronutrient intake of US athletes compared with the general population and recommendations made for athletes. *Am J Clin Nutr, 49*(5 Suppl), 1070-1076

73. Grant, S. M., Green, H. J., Phillips, S. M., & Sutton, J. R. (1997). Effects of acute expansion of plasma volume on cardiovascular and thermal function during prolonged exercise. *Eur J Appl Physiol Occup Physiol, 76*(4), 356-362

74. Green, A. L., Hultman, E., Macdonald, I. A., Sewell, D. A., & Greenhaff, P. L. (1996). Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *Am J Physiol, 271*(5 Pt 1), E821-826

75. Green, G. A., Catlin, D. H., & Starcevic, B. (2001). Analysis of over-the-counter dietary supplements. *Clin J Sport Med, 11*(4), 254-259

76. Greenstein, J. P., Birnbaum, S. M., Gullino, P., Otey, M. C., & Winitz, M. (1956). Studies on the metabolism of amino acids and related compounds in vivo. III. Prevention of ammonia toxicity by arginine and related compounds. *Arch Biochem Biophys, 64*(2), 342-354

77. Hamilton, B. (2000). East African running dominance: what is behind it? *Br J Sports Med, 34*(5), 391-394

78. Hancock, P. A., & Vasmatzidis, I. (2003). Effects of heat stress on cognitive performance: the current state of knowledge. *Int J Hyperthermia, 19*(3), 355-372

79. Harris, R. C., Soderlund, K., & Hultman, E. (1992). Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin Sci (Lond), 83*(3), 367-374

80. Hebestreit, H., Dunstheimer, D., Staschen, B., & Strassburg, H. M. (1999). Single-leg Wingate Test in children: reliability and optimal braking force. *Med Sci Sports Exerc, 31*(8), 1218-1225

81. Hew-Butler, T., Ayus, J. C., Kipps, C., Maughan, R. J., Mettler, S., Meeuwisse, W. H., Page, A. J., Reid, S. A., Rehrer, N. J., Roberts, W. O., Rogers, I. R., Rosner, M. H., Siegel, A. J., Speedy, D. B., Stuempfle, K. J., Verbalis, J. G., Weschler, L. B., & Wharam, P. (2008) Statement of the Second International Exercise-Associated Hyponatremia Consensus Development Conference, New Zealand, 2007. *Clin J Sport Med,* 18, 111-121

82. Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT: The composition of foods. Fifth revised and extended edition of McCance RA, Widdowson ED: Cambridge, UK; 1991.

83. Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. *Sports Med, 30*(1), 1-15

84. Hopkins, W. G., Hawley, J. A., & Burke, L. M. (1999). Design and analysis of research on sport performance enhancement. *Med Sci Sports Exerc, 31*(3), 472-485

85. Hopkins, W. G., Schabort, E. J., & Hawley, J. A. (2001). Reliability of power in physical performance tests. *Sports Med, 31*(3), 211-234

86. IAAF (2007). International Association of Athletics Federations: Retrieved July 5, 2010 from: http://www.iaaf.org/mm/document/imported/38451.pdf

87. IAAF (2008). International Association of Athletics Federations: Retrieved January 3, 2010 from: http://www.iaaf.org.

88. Jeevanandam, M., Ali, M. R., Holaday, N. J., Weis, J. K., & Petersen, S. R. (1993). Relative nutritional efficacy of arginine and ornithine salts of alpha-ketoisocaproic acid in traumatized rats. *Am J Clin Nutr, 57*(6), 889-896

89. Jones, A. (2006). The physiology of the world record holder for the women's marathon. *Intern J Sports Sci Coaching, 1*(2), 101-116

90. Jones, A. M., & Doust, J. H. (1996). A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. *J Sports Sci*, *14*, 321-7.

91. Kay, D. & Marino, F. E. (2003). Failure of fluid ingestion to improve self-paced exercise performance in moderate-to-warm humid environments. *Journal of Thermal Biology, 28*, 29-34

92. Kern, M., Podewils, L. J., Vukovich, M., & M.J., B. (2001). Physiological response to exercise in the heat following creatine supplementation *JEPonline 4*, 18-27

93. Kilduff, L. P., Georgiades, E., James, N., Minnion, R. H., Mitchell, M., Kingsmore, D., Hadjicharlambous, M., & Pitsiladis, Y. P. (2004). The effects of creatine supplementation on cardiovascular, metabolic, and thermoregulatory responses during exercise in the heat in endurance-trained humans. *Int J Sport Nutr Exerc Metab, 14*(4), 443-460

94. Kilduff, L. P., Pitsiladis, Y. P., Tasker, L., Attwood, J., Hyslop, P., Dailly, A., Dickson, I., & Grant, S. (2003). Effects of creatine on body composition and strength gains after 4 weeks of resistance training in previously nonresistance-trained humans. *Int J Sport Nutr Exerc Metab, 13*(4), 504-520

95. Klissouras, V. (1971). Heritability of adaptive variation. *J Appl Physiol, 31*(3), 338-344

96. Kozlowski, S., Brzezinska, Z., Kruk, B., Kaciuba-Uscilko, H., Greenleaf, J. E., & Nazar, K. (1985). Exercise hyperthermia as a factor limiting physical performance: temperature effect on muscle metabolism. *J Appl Physiol, 59*(3), 766-773

97. Kreider, R. B., Wilborn, C. D., Taylor, L., Campbell, B., Almada, A. L., Collins, R., Cooke, M., Earnest, C. P., Greenwood, M., Kalman, D. S., Kerksick, C. M., Kleiner, S. M.,

Leutholtz, B., Lopez, H., Lowery, L. M., Mendel, R., Smith, A., Spano, M., Wildman, R., Willoughby, D. S., Ziegenfuss, T. N., & Antonio, J. (2004). ISSN exercise & sport nutrition review: research & recommendations. *J Int Soc Sports Nutr, 7*, 7

98. Latzka, W. A., Sawka, M. N., Montain, S. J., Skrinar, G. S., Fielding, R. A., Matott, R. P., & Pandolf, K. B. (1998). Hyperhydration: tolerance and cardiovascular effects during uncompensable exercise-heat stress. *J Appl Physiol, 84*(6), 1858-1864

99. Lissner, L., Heitmann, B. L., & Lindroos, A. K. (1998). Measuring intake in free-living human subjects: a question of bias. *Proc Nutr Soc, 57*(2), 333-339

100. Liu, T. H., Wu, C. L., Chiang, C. W., Lo, Y. W., Tseng, H. F., & Chang, C. K. (2009). No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. *J Nutr Biochem, 20*(6), 462-468

101. Lyons, T. P., Riedesel, M. L., Meuli, L. E., & Chick, T. W. (1990). Effects of glycerol-induced hyperhydration prior to exercise in the heat on sweating and core temperature. *Med Sci Sports Exerc, 22*(4), 477-483

102. MacDougall, J. D., Reddan, W. G., Layton, C. R., & Dempsey, J. A. (1974). Effects of metabolic hyperthermia on performance during heavy prolonged exercise. *J Appl Physiol, 36*(5), 538-544

103. MacLean, D. A., Graham, T. E., & Saltin, B. (1996). Stimulation of muscle ammonia production during exercise following branched-chain amino acid supplementation in humans. *J Physiol, 493 (Pt 3)*, 909-922

104. Madsen, K., MacLean, D. A., Kiens, B., & Christensen, D. (1996). Effects of glucose, glucose plus branched-chain amino acids, or placebo on bike performance over 100 km. *J Appl Physiol, 81*(6), 2644-2650

105. Magal, M., Webster, M. J., Sistrunk, L. E., Whitehead, M. T., Evans, R. K., & Boyd, J. C. (2003). Comparison of glycerol and water hydration regimens on tennis-related performance. *Med Sci Sports Exerc, 35*(1), 150-156

106. Marcell, T. J., Taaffe, D. R., Hawkins, S. A., Tarpenning, K. M., Pyka, G., Kohlmeier, L., Wiswell, R. A., & Marcus, R. (1999). Oral arginine does not stimulate basal or augment exercise-induced GH secretion in either young or old adults. *J Gerontol A Biol Sci Med Sci, 54*(8), M395-399

107. Marfell-Jones, M., Olds, T., Stewart, A., Carter, L. International Standards for Anthropometric Assessment. International Society for the Advancement of Kinanthropometry ISAK, (2nd Ed) Potchefstroom; 2006

108. Marino, F. E., Mbambo, Z., Kortekaas, E., Wilson, G., Lambert, M. I., Noakes, T. D., & Dennis, S. C. (2000). Advantages of smaller body mass during distance running in warm, humid environments. *Pflugers Arch, 441*(2-3), 359-367

109. Maughan, R. J. (1982). A simple, rapid method for the determination of glucose, lactate, pyruvate, alanine, 3-hydroxybutyrate and acetoacetate on a single 20-mul blood sample. *Clin Chim Acta, 122*(2), 231-240

110. Maughan, R. J., King, D. S., & Lea, T. (2004). Dietary supplements. *J Sports Sci, 22*(1), 95-113

111. Maughan, R. J., Shirreffs, S. M., & Leiper, J. B. (2007). Errors in the estimation of hydration status from changes in body mass. *J Sports Sci, 25*, 797-804.

112. McConell, G. K., Burge, C. M., Skinner, S. L., & Hargreaves, M. (1997). Influence of ingested fluid volume on physiological responses during prolonged exercise. *Acta Physiol Scand, 160*(2), 149-156

113. McConell, G. K., Huynh, N. N., Lee-Young, R. S., Canny, B. J., & Wadley, G. D. (2006). L-Arginine infusion increases glucose clearance during prolonged exercise in humans. *Am J Physiol Endocrinol Metab, 290*(1), E60-E66

114. Meneguello, M. O., Mendonca, J. R., Lancha, A. H., Jr., & Costa Rosa, L. F. (2003). Effect of arginine, ornithine and citrulline supplementation upon performance and metabolism of trained rats. *Cell Biochem Funct, 21*(1), 85-91

115. Meredith, C. N., Zackin, M. J., Frontera, W. R., & Evans, W. J. (1989). Dietary protein requirements and body protein metabolism in endurance-trained men. *J Appl Physiol, 66*(6), 2850-2856

116. Meyer, R. A., & Terjung, R. L. (1979). Differences in ammonia and adenylate metabolism in contracting fast and slow muscle. *Am J Physiol, 237*(3), C111-118

117. Millard-Stafford, M., Sparling, P. B., Rosskopf, L. B., Snow, T. K., DiCarlo, L. J., & Hinson, B. T. (1995). Fluid intake in male and female runners during a 40-km field run in the heat. *J Sports Sci, 13*(3), 257-263

118. Minuskin, M. L., Lavine, M. E., Ulman, E. A., & Fisher, H. (1981). Nitrogen retention, muscle creatine and orotic acid excretion in traumatized rats fed arginine and glycine enriched diets. *J Nutr, 111*(7), 1265-1274

119. Montain, S. J., Cheuvront, S. N., & Sawka, M. N. (2006). Exercise associated hyponatraemia: quantitative analysis to understand the aetiology. *Br J Sports Med, 40*(2), 98-105; discussion 198-105

120. Montain, S. J., & Coyle, E. F. (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol, 73*(4), 1340-1350

121. Montain, S. J., & Coyle, E. F. (1993). Influence of the timing of fluid ingestion on temperature regulation during exercise. *J Appl Physiol, 75*(2), 688-695

122. Montain, S. J., Latzka, W. A., & Sawka, M. N. (1999). Fluid replacement recommendations for training in hot weather. *Mil Med, 164*(7), 502-508

123. Moore, R. A., Derry, S., & Aldington, D. (2011). From evidence-based medicine to guidelines and recommendations: a long and winding road. *Eur J Anaesthesiol, 28*(11), 753-755

124. Moses, K., & Manore, M. M. (1991). Development and testing of a carbohydrate monitoring tool for athletes. *J Am Diet Assoc, 91*(8), 962-965.

125. Muir, A. L., Percy-Robb, I. W., Davidson, I. A., Walsh, E. G., & Passmore, R. (1970). Physiological aspects of the Edinburgh commonwealth games. *Lancet, 2*(7683), 1125-1128

126. Mukeshi, M., & Thairu, K. (1993). Nutrition and body build: a Kenyan review. *World Rev Nutr Diet, 72*, 218-226

127. Murray, R., Eddy, D. E., Paul, G. L., Seifert, J. G., & Halaby, G. A. (1991). Physiological responses to glycerol ingestion during exercise. *J Appl Physiol, 71*(1), 144-149

128. Myburgh, K. H. (2003). What makes an endurance athlete world-class? Not simply a physiological conundrum. *Comp Biochem Physiol A Mol Integr Physiol, 136*(1), 171-190

129. Myers, M. J., & Steudel, K. (1985). Effect of limb mass and its distribution on the energetic cost of running. *J Exp Biol, 116*, 363-373

130. Nadel, E. R., Fortney, S. M., & Wenger, C. B. (1980). Effect of hydration state of circulatory and thermal regulations. *J Appl Physiol, 49*(4), 715-721

131. Nair, K. S., Schwartz, R. G., & Welle, S. (1992). Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol, 263*(5 Pt 1), E928-934

132. National Research Council. In: Recommended Dietary Allowances. DC Press: National Academy, Washington., 249; 1989

133. Nelson, J. L., & Robergs, R. A. (2007). Exploring the potential ergogenic effects of glycerol hyperhydration. *Sports Med, 37*(11), 981-1000

134. Nielsen, B. (1996). Olympics in Atlanta: A fight against physics. *Med Sci Sports Exerc, 28,* 665-8.

135. Nielsen, B., Hales, J. R., Strange, S., Christensen, N. J., Warberg, J., & Saltin, B. (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *J Physiol, 460*, 467-485

136. Noakes, T. D. (2001). Temperature regulation during exercise. The Lore of Running. 4th ed. Champaign, IL: Human Kinetics.

137. Noakes, T. D. (2003). Fluid replacement during marathon running. *Clin J Sport Med, 13*(5), 309-318

138. Noakes, T. D. (1993). Fluid replacement during exercise. *Exerc Sport Sci Rev, 21*, 297-330

139. Noakes, T. D. (2003). Overconsumption of fluids by athletes. *BMJ, 327*(7407), 113-114

140. Noakes, T. D. (2007a). Drinking guidelines for exercise: what evidence is there that athletes should drink "as much as tolerable", "to replace the weight lost during exercise" or "ad libitum"? *J Sports Sci, 25*(7), 781-796

141. Noakes, T. D. (2007b). Hydration in the marathon : using thirst to gauge safe fluid replacement. *Sports Med, 37*(4-5), 463-466

142. Noakes, T. D., Adams, B. A., Myburgh, K. H., Greeff, C., Lotz, T., & Nathan, M. (1988). The danger of an inadequate water intake during prolonged exercise. A novel concept re-visited. *Eur J Appl Physiol Occup Physiol, 57*(2), 210-219

143. Noakes, T. D., Sharwood, K., Speedy, D., Hew, T., Reid, S., Dugas, J., Almond, C., Wharam, P., & Weschler, L. (2005). Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2,135 weighed competitive athletic performances. *Proc Natl Acad Sci U S A, 102*(51), 18550-18555

144. Nolte, H. W., Noakes, T. D., & van Vuuren, B. (2011). Protection of total body water content and absence of hyperthermia despite 2% body mass loss ('voluntary dehydration') in soldiers drinking ad libitum during prolonged exercise in cool environmental conditions. *Br J Sports Med, 45,* 1106-12.

145. Nose, H., Mack, G. W., Shi, X. R., & Nadel, E. R. (1988). Shift in body fluid compartments after dehydration in humans. *J Appl Physiol, 65*(1), 318-324

146. O'Brien, C., Young, A. J., & Sawka, M. N. (2002). Bioelectrical impedance to estimate changes in hydration status. *Int J Sports Med, 23*(5), 361-366

147. Onywera, V. O., Kiplamai, F. K., Boit, M. K., & Pitsiladis, Y. P. (2004). Food and macronutrient intake of elite kenyan distance runners. *Int J Sport Nutr Exerc Metab, 14*(6), 709-719

148. Onywera, V. O., Scott, R. A., Boit, M. K., & Pitsiladis, Y. P. (2006). Demographic characteristics of elite Kenyan endurance runners. *J Sports Sci, 24*(4), 415-422

149. Pastene, J., Germain, M., Allevard, A. M., Gharib, C., & Lacour, J. R. (1996). Water balance during and after marathon running. *Eur J Appl Physiol Occup Physiol, 73*(1-2), 49-55.

150. Pitkanen, H. T., Oja, S. S., Rusko, H., Nummela, A., Komi, P. V., Saransaari, P., Takala, T., & Mero, A. A. (2003). Leucine supplementation does not enhance acute strength or running performance but affects serum amino acid concentration. *Amino Acids, 25*(1), 85-94

151. Pitsiladis, Y., & Wang, G. (2011). Necessary advances in exercise genomics and likely pitfalls. *J Appl Physiol, 110*(5), 1150-1151

152. Poortmans, J. R., Rawson, E. S., Burke, L. M., Stear, S. J., & Castell, L. M. (2011). A-Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance Part 11. *Br J Sports Med, 44*(10), 765-766

153. Pugh, L. G., Corbett, J. L., & Johnson, R. H. (1967). Rectal temperatures, weight losses, and sweat rates in marathon running. *J Appl Physiol, 23*(3), 347-352

154. Riedesel, M. L., Allen, D. Y., Peake, G. T., & Al-Qattan, K. (1987). Hyperhydration with glycerol solutions. *J Appl Physiol, 63*(6), 2262-2268

155. Rodriguez, N. R., Di Marco, N. M., & Langley, S. (2009). American College of Sports Medicine position stand. Nutrition and athletic performance. *Med Sci Sports Exerc, 41*(3), 709-731

156. Sandstedt, S., Jorfeldt, L., & Larsson, J. (1992). Randomized, controlled study evaluating effects of branched chain amino acids and alpha-ketoisocaproate on protein metabolism after surgery. *Br J Surg, 79*(3), 217-220

157. Saunders, P., Dugas, J. P., Tucker, R., Lambert, M. I., & Noakes, T. D. (2005). The effects of different air velocities on heat storage and body temperature in humans cycling in a hot, humid environment. *Acta Physiol Scand, 183*(3), 241-255

158. Saunders, P., Pyne, D. B., Telford, R. D., & Hawley, J. A. (2004). Factors affecting running economy in trained distance runners. *Sports Med, 34*(7), 465-485

159. Sawka, M. N. (1992). Physiological consequences of hypohydration: exercise performance and thermoregulation. *Med Sci Sports Exerc, 24*(6), 657-670

160. Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc, 39*(2), 377-390

161. Sawka, M. N., & Montain, S. J. (2000). Fluid and electrolyte supplementation for exercise heat stress. *Am J Clin Nutr, 72*(2 Suppl), 564S-572S

162. Sawka, M. N., Montain, S. J., & Latzka, W. A. (2001). Hydration effects on thermoregulation and performance in the heat. *Comp Biochem Physiol A Mol Integr Physiol, 128*(4), 679-690

163. Sawka, M. N., & Noakes, T. D. (2007). Does dehydration impair exercise performance? *Med Sci Sports Exerc, 39*(8), 1209-1217

164. Schabort, E. J., Hawley, J. A., Hopkins, W. G., & Blum, H. (1999). High reliability of performance of well-trained rowers on a rowing ergometer. *J Sports Sci, 17*(8), 627-632

165. Schena, F., Guerrini, F., Tregnaghi, P., & Kayser, B. (1992). Branched-chain amino acid supplementation during trekking at high altitude. The effects on loss of body mass, body composition, and muscle power. *Eur J Appl Physiol Occup Physiol, 65*(5), 394-398

166. Schofield, W. N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr, 39 Suppl 1*, 5-41

167. Scott, R. A., Fuku, N., Onywera, V. O., Boit, M., Wilson, R. H., Tanaka, M., Goodwin, W. H., & Pitsiladis, Y. P. (2009). Mitochondrial haplogroups associated with elite Kenyan athlete status. *Med Sci Sports Exerc, 41*(1), 123-128

168. Scott, R. A., Georgiades, E., Wilson, R. H., Goodwin, W. H., Wolde, B., & Pitsiladis, Y. P. (2003). Demographic characteristics of elite Ethiopian endurance runners. *Med Sci Sports Exerc, 35*(10), 1727-1732

169. Scott, R. A., & Pitsiladis, Y. P. (2007). Genotypes and distance running : clues from Africa. *Sports Med, 37*(4-5), 424-427

170. Sharwood, K. A., Collins, M., Goedecke, J. H., Wilson, G., & Noakes, T. D. (2004). Weight changes, medical complications, and performance during an Ironman triathlon. *Br J Sports Med, 38*(6), 718-724

171. Sitren, H. S., & Fisher, H. (1977). Nitrogen retention in rats fed on diets enriched with arginine and glycine. 1. Improved N retention after trauma. *Br J Nutr, 37*(2), 195-208

172. Stead, L. M., Au, K. P., Jacobs, R. L., Brosnan, M. E., & Brosnan, J. T. (2001). Methylation demand and homocysteine metabolism: effects of dietary provision of creatine and guanidinoacetate. *Am J Physiol Endocrinol Metab, 281*(5), E1095-1100

173. Steenge, G. R., Simpson, E. J., & Greenhaff, P. L. (2000). Protein- and carbohydrate-induced augmentation of whole body creatine retention in humans. *J Appl Physiol, 89*(3), 1165-1171

174. Stevens, B. R., Godfrey, M. D., Kaminski, T. W., & Braith, R. W. (2000). High-intensity dynamic human muscle performance enhanced by a metabolic intervention. *Med Sci Sports Exerc, 32*(12), 2102-2108

175. Taylor, R., & Giles, J. (2005). Cash interests taint drug advice. *Nature, 437*(7062), 1070-1071

176. Terjung, R. L., Dudley, G. A., & Meyer, R. A. (1985). Metabolic and circulatory limitations to muscular performance at the organ level. *J Exp Biol, 115*, 307-318

177. van Erp-Baart, A. M., Saris, W. H., Binkhorst, R. A., Vos, J. A., & Elvers, J. W. (1989). Nationwide survey on nutritional habits in elite athletes. Part I. Energy, carbohydrate, protein, and fat intake. *Int J Sports Med, 10 Suppl 1*, S3-10

178. van Hall, G., Raaymakers, J. S., Saris, W. H., & Wagenmakers, A. J. (1995). Ingestion of branched-chain amino acids and tryptophan during sustained exercise in man: failure to affect performance. *J Physiol, 486 (Pt 3)*, 789-794

179. van Hall, G., van der Vusse, G. J., Soderlund, K., & Wagenmakers, A. J. (1995). Deamination of amino acids as a source for ammonia production in human skeletal muscle during prolonged exercise. *J Physiol, 489 (Pt 1)*, 251-261

180. Van Rooyen, M., Hew-Butler, T. D., & Noakes, T. D. (2010). Drinking during marathon running in extreme heat: a video analysis study of the top finishers in the 2004 Athens Olympic marathons. *SA J Sport Med. , 22*, 55-61

181. Van Rosendal, S. P., Osborne, M. A., Fassett, R. G., & Coombes, J. S. (2010). Guidelines for glycerol use in hyperhydration and rehydration associated with exercise. *Sports Med, 40*(2), 113-129

182. Vandenberghe, K., Gillis, N., Van Leemputte, M., Van Hecke, P., Vanstapel, F., & Hespel, P. (1996). Caffeine counteracts the ergogenic action of muscle creatine loading. *J Appl Physiol, 80*(2), 452-457

183. Verbalis, J. G. (2003). Disorders of body water homeostasis. *Best Pract Res Clin Endocrinol Metab, 17*(4), 471-503

184. Volek, J. S., Mazzetti, S. A., Farquhar, W. B., Barnes, B. R., Gomez, A. L., & Kraemer, W. J. (2001). Physiological responses to short-term exercise in the heat after creatine loading. *Med Sci Sports Exerc, 33*(7), 1101-1108

185. WADA (2010). World Anti-Doping Agency. Prohibited List Retrieved Nov 21, 2011 from http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf.

186. Wagenmakers, A. J. (1999). Amino acid supplements to improve athletic performance. *Curr Opin Clin Nutr Metab Care, 2*(6), 539-544

187. Walker, J. B. (1979). Creatine: biosynthesis, regulation, and function. *Adv Enzymol Relat Areas Mol Biol, 50*, 177-242

188. Ward MP, Milledge JS, West JB. High Altitude Medicine and Physiology. Chapman & Hall Medical, London; 1995.

189. Watt, M. J., Garnham, A. P., Febbraio, M. A., & Hargreaves, M. (2000). Effect of acute plasma volume expansion on thermoregulation and exercise performance in the heat. *Med Sci Sports Exerc, 32*(5), 958-962

190. Weimann, A., Bastian, L., Bischoff, W. E., Grotz, M., Hansel, M., Lotz, J., Trautwein, C., Tusch, G., Schlitt, H. J., & Regel, G. (1998). Influence of arginine, omega-3 fatty acids and nucleotide-supplemented enteral support on systemic inflammatory response syndrome and multiple organ failure in patients after severe trauma. *Nutrition, 14*(2), 165-172

191. Westerterp-Plantenga, M. S., Rolland, V., Wilson, S. A., & Westerterp, K. R. (1999). Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber. *Eur J Clin Nutr, 53*(6), 495-502

192. Wharam, P. C., Speedy, D. B., Noakes, T. D., Thompson, J. M., Reid, S. A., & Holtzhausen, L. M. (2006). NSAID use increases the risk of developing hyponatremia during an Ironman triathlon. *Med Sci Sports Exerc, 38*(4), 618-622

193. Wilkinson, D. J., Smeeton, N. J., & Watt, P.W. (2010). Ammonia metabolism, the brain and fatigue; revisiting the link. *Prog Neurobiol*, *91*(3), 200-19. Epub 2010 Feb 4

194. Williams, A. G., & Folland, J. P. (2008). Similarity of polygenic profiles limits the potential for elite human physical performance. *J Physiol, 586*(1), 113-121

195. Wyndham, C. H., & Strydom, N. B. (1969). The danger of an inadequate water intake during marathon running. *S Afr Med J, 43*(29), 893-896

196. Yarrow, J. F., Parr, J. J., White, L. J., Borsa, P. A., & Stevens, B. R. (2007). The effects of short-term alpha-ketoisocaproic acid supplementation on exercise performance: a randomized controlled trial. *J Int Soc Sports Nutr, 4*, 2

197. Zouhal, H., Groussard, C., Minter, G., Vincent, S., Cretual, A., Gratas-Delamarche, A., Delamarche, P., & Noakes, T. D. (2010). Inverse relationship between percentage body weight change and finishing time in 643 forty-two-kilometre marathon runners. *Br J Sports Med, 45*(14), 1101-1105

198. Zouhal, H., Groussard, C., Vincent, S., Jacob, C., Abderrahman, A. B., Delamarche, P., & Gratas-Delamarche, A. (2009). Athletic performance and weight changes during the "Marathon of Sands" in athletes well-trained in endurance. *Int J Sports Med, 30*(7), 516-521