

# THE KETONE FILES



Page 2 – Participation consent form

Page 3 – UK Sport confidential memo to board proposing the secret ketone project before London 2012

Page 17 – Presentation by Scott Drawer, UK Sport Head of Innovation, later Team Sky, on ketone performance-enhancing effects

Page 39 – UK Sport Project Application form

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Page 59 – 2017 PHD study reviewing the UK Sport ketone programme in detail. Ketones were given to 91 British athletes in the lead up to London 2012.

Page 245 – Delta G invoices to UK Sport

Page 249 – Presentation by Delta G (ketones) creator, including slide stating with Sir Bradley Wiggins was one of the “early adopters”

## Participant Consent Form

### The effects of supplemental ketosis on [REDACTED] [REDACTED] performance

1. I have read the information sheet, which provides full details of this study, and have had the opportunity to raise and discuss my questions with a named researcher regarding the general nature, object, potential risks and duration of the study, and understand what is expected of me.
2. I understand that the aim of the study is to investigate the effects of a specifically formulated dietary supplement on [REDACTED] [REDACTED] performance.
3. I agree to volunteer as a participant for the study described in the information sheet. I give my full consent to my participation in this study.
4. I confirm that I do not know of any medical condition that I suffer from which might have the effect of making it more likely that I be involved in an incident which could result in injury to myself or others.
5. As part of my agreement to participate I accept that there are risks in this study due to performing maximal exercise and to ingesting the ketone ester supplement. I accept that I cannot hold those running or associated with this protocol or their respective institutes, associations and businesses at any liability for these risks.
6. I consent to UK Sport and the project team members processing my data under the Data Protection Act 1998 for the purpose of this study only and for no other purpose whatsoever. I consent that UK Sport and the project team members may share my data unanonymised to [REDACTED] for the purposes of assessing my performance under the study. Any other presentation of my own individual data outside of [REDACTED] will maintain my anonymity unless I specifically give my permission otherwise.
7. I reserve the right to withdraw from this experiment at any time without giving a reason; I understand that I may be withdrawn at any time, and will suffer no penalty as a result.

\_\_\_\_\_  
Name of participant

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Name of investigator

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

## **CONFIDENTIAL**

BOARD MEMBERS AND DIRECTORS TEAM ONLY

### **A NOVEL FOOD SOURCE THAT IMPROVES ENDURANCE PERFORMANCE THROUGH A SUPPLEMENT-INDUCED KETOSIS**

#### **Background**

1. In 2007, UK Sport R&I team picked up on a short article 'Superhuman' in the New Scientist (25 July, 2007, issue 2614). The article outlined some background work on a new nutritional food source (DeltaG) which had been developed with US military and was being tested in animals for physical and cognitive endurance performance.
2. Initial contact to the US (Defence Advanced Research Projects Agency DARPA) led back to the UK to ██████████ at Oxford University (spinout company - TDelta). At the time, the work was going through animal testing. Before it could be considered for any human trials, numerous safety, toxicity and food / drug approvals were required. The initial work on animals showed significant performance advantages with a single dose of DeltaG.
3. In 2009 / 2010, all safety and federal approvals (US) were confirmed. In addition, the Medicines and Healthcare products Regulatory Association (UK, <http://www.mhra.gov.uk/index.htm>) have confirmed that this is neither a food nor a pharmaceutical. At this point, UK Sport re-engaged to help Oxford University structure some initial lab based experimental performance work with humans. Recently (Sept, 2011), UKAD and WADA has also confirmed that use of this novel product does not contravene current anti-doping regulations (see appendix 1); and an independent scientific panel has verified the science behind the concept (see appendix 2).
4. Human performance trials have shown significant performance improvements in a 30 minute fixed duration rowing effort in national / international athletes. The majority of participating athletes improved within a 1-2% margin. One athlete broke the WR for this timed trial and numerous achieved Season Best's and Personal Best's.
5. In a further experimental trial looking at repeat power performance in a mixed demand sport model over 90 minutes, an additional statistically significant improvement in repeat power performance (2%) was seen when compared to a standard carbohydrate drink taken pre performance.
6. Further experimental work is proposed with the purpose to strengthen the performance evidence to answer further questions related to the optimal dosing and potential impact across different exercise durations and modes.
7. Subject to the outcomes from these further experimental and applied trials, the aim is to implement the use of DeltaG with targeted athletes and sports in the period leading into and during London 2012 with events greater than 5 minutes duration and multi event athletes. These sports include cycling; hockey; sailing; athletics; swimming; modern pentathlon and select others. Subject to further evidence being generated there could also be value to rowing, boxing, taekwondo, judo and targeted Paralympic athletes.

## Current landscape

8. UK Sport is currently negotiating an exclusive agreement with Professor [REDACTED] and the spinout company which owns the patents and rights to DeltaG with an eye to use with targeted athletes and sports in London 2012.
9. Currently, DeltaG is only available to UK Sport and only for the purpose of 'research' – it is not commercially available to anyone else. The only people who are currently allowed to purchase and use the product outside the context of research trials are the US Special Forces.
10. As this situation is highly unlikely to change before London 2012, all usage of DeltaG has to happen under the umbrella of a scientific experiments, including the provision of formal ethical approval. This also means that Team GB and ParalympicsGB will, most likely, be the only nation in 2012 who is aware of and able to utilise the performance effects DeltaG offers.

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
12. There are some critical 'parallel' steps required in the next 8 months to ensure an effective roll out prior to London 2012. More experimental evidence is beginning to emerge on the impact of DeltaG above and beyond current nutritional practice prior to an acute competitive event. In no particular order, these include:
    - a. Confirmation of further experimental and applied evidence of the performance impact and general tolerance of DeltaG when used by GB Olympic and Paralympic athletes under competition conditions – **April 2012**. UK Sport R&I will absorb costs with further research through existing research teams. Estimated costs for this will be £225k (incl. VAT).
    - b. Gain sufficient ethical approval to allow the usage of DeltaG by targeted British athletes to establish the performance impact and general tolerance of DeltaG (see previous point) as well as during the Olympic and Paralympic Games in 2012. UK Sport R&I is currently preparing an ethics application for submission before the end of **October 2011**.
    - c. Up scaling of the required volume for future testing and roll out leading into and during London 2012. Maximum costs to develop the volume required prior to London 2012 across a number of targeted athletes and sports are £200k (incl. VAT). **Sports will be expected to contribute to these final costs once final research and case study work has been completed (Paragraph 12a above).**
    - d. Communication process with targeted sports where there is likely to be a performance opportunity. This has to be done under extreme confidentiality linking with sports current technical staff and in partnership with BOA – BPA during Games time for effective control and management. The current strategy is NOT to talk about the project. However, a communication plan will be needed with the media to mitigate any negative perceptions and publicity. The publicity focus will need to be on the quality of science, the quality of research partner and the fact that the UK is ahead of the curve when compared to its competitors.
    - e. Board approval to continue pursuing this opportunity on the basis if the required investment to get to London 2012.



13. Subject to all steps above being achieved, UK Sport is seeking board approval to pursue to the next phase and commit to the resources required to achieve tasks in paragraphs 12a. to 12d. above.

## Risks

14. There are potential risks of formally partnering with TDelta and Oxford University. These include:
  - a. **Athletes right to be informed** of DeltaG before using it and the status of it in the context of the anti-doping regime. Athletes should also be informed of its contents and any potential side effects in order to undertake their own checks against it. For example Athlete may wish to check DeltaG ingredients by utilising the Drug Reference Online database administered by UKAD. Athletes should not feel they are obliged to participate or forced into participating in the use of DeltaG and those that choose to participate in the case studies should feel free to stop their participation and use of DeltaG. These points, i.e. the emphasis on the informed consent to participate in this opportunity in light of any potential risks as well as the voluntary nature of the participation with the right to withdraw at any point, will be considered in the ethical consent process.
  - b. **Confidentiality management** with sports. Management of the information will be done via standard legal documents and targeted information sharing.
  - c. **Publicity and risk communication** during time of games of this GB exclusive nutritional strategy. The focus will need to be on the science quality, the underlying mechanisms and impact for society.
  - d. Magnitude of **available performance evidence** relevant to current nutritional practice. The focus for London is on acute performance and there should be sufficient confidence at this stage to make solid recommendations. Beyond London there are further opportunities to investigate application across training and recovery models in sport.
  - e. **Manufacture process** and quality assurance of delivered product. Although this can be tested and controlled based on current best practice, the choice for use must remain with the athlete. UK Sport would adhere to UKAD recommendations using HFL to test and review manufacture line.
  - f. **Product taste and tolerability.** It is fair to say that the current prototype product is not that pleasant but tolerable for most people. TDelta are currently investigating various food technologies to improve taste and food delivery and it will be crucial for implementation. UK Sport will furthermore emphasise in the discussions with the sports that a main focus of the initial roll out phase needs to be to confirm the tolerability of each targeted athlete wanting to use the product before using it at the Olympics and Paralympics.
  - g. **WADA might exercise their reserved rights to regulate as stated in UKAD's letter collected blood samples or retrospectively test old samples.** This may occur if there is pressure of the media prior to 2012 if the concept was to leak. However, WADA have already published the prohibited list for London 2012. In addition, ketosis is a temporary physiological state and would be very difficult to prove or test with any post event samples as the fuel source is utilised during exercise.
  - h. **Hypothesis testing at the Olympic and Paralympic Games.** As the product is only available for 'research' purposes the use at the London 2012 OG / PGs will require formal ethical approval based on standard scientific protocols. UKS needs to be successful in the application for sufficient ethical approval and athletes will have to sign informed consent and NDAs to manage the process.

15. Overall, it appears the risks are manageable based on current best practice and available knowledge.

16. In addition, the communication and release of information into the public domain needs significant consideration. TDelta do need to release scientific data into the public domain through academic publications for future commercial application – this needs to occur at the right time and with the right content to support the project in an effective way. However, this will raise interest from the international community who could look at ways of responding. Although the product cannot be obtained, if others are aware UK Sport is linked into the project, the risks will be around a perceived unfair competitive edge. Current proposals are looking to ensure any formal scientific publications are only released post London 2012. However, it should be noted that UK Sport association with this project and University could also provide credibility to the scientific story.

### **Options for consideration**

17. There are two options for consideration:

1. Do not pursue the project.
2. Continue the project in a phased way (part a November 2011 to April 2012; part b April 2012 to September 2012) to manage all risks with significant communications support to manage information release and product implementation.

### **Recommendations**

18. To maximise the opportunities and minimise risks for UK Sport, Board is asked to adopt Option 2 subject to:

- a. HFL quality assurance of the product manufacture line;
- b. An effective agreed communication strategy with BOA, BPA and NGBs;
- c. Resourcing from within the UKS Performance budgets;
- d. Commitment from GB sports demonstrated by financial resourcing to manufacture and product costs and a formal contract in pace with UKS;
- e. Development of recommended performance evidence for acute use during a single competitive tournament;
- f. An approved ethics proposal supporting the work at the Olympic and Paralympic Games.

19. Due to the value of the agreement with TDelta authority falls within the CEO's delegated authority. However, due to the circumstances oversight and specific authority is sought from Board to enter into the agreement with TDelta.

  
October 2011

## Appendix 1 – UKAD and WADA review

**Protect**

[REDACTED]  
UK Sport  
40 Bernard Street  
London  
WC1N 1ST

13 October 2011

Dear [REDACTED]

Re: [REDACTED]

This letter details the processes followed with respect to the research conducted by Oxford University, as headed up by [REDACTED] regarding the Ketone dietary supplement [REDACTED]

The correspondence was initiated with UK Anti-Doping on the prohibited status of Ketones back in April of this year, shortly thereafter (11/04/2011) we met to discuss this topic. At this point only information in the public domain was exchanged (New Scientist article, 25/07/2007) due to the confidentiality agreement (CDA) between UK Sport and Oxford University. Until this initial meeting, and the follow up emails that took place between UK Anti-Doping, the World Anti-Doping Agency (WADA) and UK Sport, it was not known what level of information would be required by UK Anti-Doping and WADA to assess the prohibited status of this Ketone as if the generic category of Ketones could be confirmed as not prohibited, then no further action was required.

The substances categorised as Ketones are diverse. To determine whether the status of [REDACTED] was prohibited or not further information regarding the substance needed to be provided to UK Anti-Doping and WADA. A CDA was therefore signed between UK Anti-Doping, Oxford University and UK Sport. [REDACTED] from Oxford University and UK Anti-Doping met in July to identify the detailed information required. On 26 July, an 18 page dossier was submitted by UK Sport and Oxford University to UK Anti-Doping and, following the signing of a CDA between WADA and Oxford University, it was forwarded to [REDACTED] the [REDACTED] at WADA.

A response received from WADA (08/09/2011) expressed no concern over this substance,

"...Frankly, none of us here in the Science Dept understand why these substances would be of particular concerns from an anti-doping perspective. We would appreciate further clarifications from you..."

UK Anti-Doping has clarified two further points with WADA and received a final response,



"At this point in time and based on the confidential information from TAS we have reviewed, we have no reason to consider such substances as banned under the 2011 List of Prohibited Substances and Methods.

Should further information or future results justify a review of this judgement, we of course reserve the right to review our current position."

I hope this information is of assistance.

Yours sincerely



█

UK Anti-Doping Security Marking: **PROTECT**

For further information regarding security marking please visit the below link.

<http://www.ukad.org.uk/pages/GPMS/>

**Appendix 2 – Independent Scientific Review Summary**

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[REDACTED]  
UK Sport  
40 Bernard Street  
London WC1N 1ST

9<sup>th</sup> October 2011

Dear [REDACTED]

INDEPENDENT REVIEW - 28<sup>TH</sup> SEPTEMBER 2011

On the 28<sup>th</sup> September a meeting was held at Loughborough University of independent experts with the aim of considering the scientific evidence associated with a novel nutritional intervention (NNI) in the context of enhancing the physical and cognitive performance of elite athletes. Those present at the meeting were:

[REDACTED]

Those marked with an asterisk were involved in the independent evaluation of the concept and data present.

For the first hour of the meeting [REDACTED] described the NNI and presented studies in which its efficacy had been tested. During the second hour of the meeting [REDACTED] answered questions on the scientific findings presented and the interpretation of these findings. In advance of the meeting papers relating to the NNI were circulated by UK Sport, included in these was the dossier prepared for the World Anti-Doping Agency (WADA).

At the end of the meeting those evaluating the scientific evidence in support of the NNI were asked to address three questions; these are presented below with a summary of their answers.

- [REDACTED]
1. *Are we happy for UK athletes to use this NNI from the perspective of safety?*  
The consensus view was that the group were happy for UK athletes to use the NNI, especially given the data presented and pre-circulated, and on the understanding that the introduction and use of the NNI will be closely monitored at the level of individual athletes. It was noted that the repeated use of the NNI in heavily exercising athletes, competing in high pressure situations and perhaps consuming other permitted products, is yet to be assessed.
  2. *Are we happy for UK athletes to use the NNI as an aid to performance?* On the basis of the evidence presented and pre-circulated, the group considered that the NNI had the potential to significantly (1-2%) enhance performance in endurance events. It is possible, but as yet not established, that the NNI may be beneficial over multiple rounds of shorter duration events (e.g. multi-sprints) and with glycogen re-synthesis ("nutrition for recovery").
  3. *What further, high priority, information or actions would you like to see in order to establish the full utility of the NNI and its optimal use?*
    - a. Ensure the NNI has an independent beneficial impact on performance by testing it on an appropriate athletic population against current best practice in terms of nutrition (e.g. glucose, caffeine etc)\*.
    - b. Assess the impact of repeated use of the NNI in heavily exercising athletes, competing in high pressure situations and perhaps consuming other permitted products\*.
    - c. Assess the benefit of the NNI over multiple rounds of shorter duration events\*.
    - d. Establish the optimal dosage of the NNI for individuals with a wide range of body weight\*.
    - e. Establish the benefit of the NNI for repeat bouts of high intensity exercise.
    - f. Determine the adaptation period required for the NNI (if any), and whether or not its use, or habituation to its use, has any detrimental effects on other metabolic pathways.
    - g. Given the data presented on the beneficial effect of the NNI on cognitive performance (in rats), consider the potential for the NNI to improve performance in sports with a high cognitive load.
    - h. Establish the benefit of the NNI for prolonged (e.g. 2 hours plus) endurance exercise\*.
    - i. Assess the effect of the NNI on glycogen re-synthesis rate following intense exercise.

Based on current evidence, it would seem reasonable to focus initial use of the NNI on endurance-related sports [REDACTED]

[REDACTED] Given the time available before the 2012 Olympics it should be feasible to achieve a small number of case studies and perhaps one or two experimental studies in order to meet the objective of supporting and developing the evidence-base for targeted athletes. Those areas of further study marked in the list above with an asterisk are thought to be able to be addressed either by experimentation (a, h); as part of the implementation plan in smaller competitions



[Redacted]

before the Olympics (b. c); or in case studies with targeted athletes (d.). The other areas may take longer to achieve and therefore be post-Olympic projects.

It was agreed by all present that the introduction of the NNI should be very carefully handled and monitored down to the level of individual athletes and coaches so that they are fully aware of how and when to use the NNI, and they have had experience using and evaluating the NNI before use in serious competition. It is understood that this is standard practice for UKSport.

As a result of the review undertaken, it was concluded that the NNI should be introduced and used in a controlled and monitored way by those athletes most likely to benefit from it, as identified by UKSport.

*Yours sincerely,*

[Redacted Signature]

[Redacted]

## Appendix 3 – Project Initiation Document (PID)

## Appendix 4 – Legal Advice

Confidential and Subject to Legal Privilege

[REDACTED]

- [REDACTED]

- [REDACTED]

- [REDACTED]

[REDACTED]

[REDACTED]

- [REDACTED]

- [REDACTED]

- [REDACTED]

[REDACTED]

- [REDACTED]

- [REDACTED]



# UK Sport R&I programme

Dr Scott Drawer

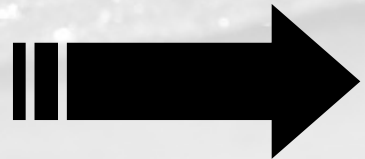
Head of Research & Innovation

UK Sport

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# UK approach to R&I

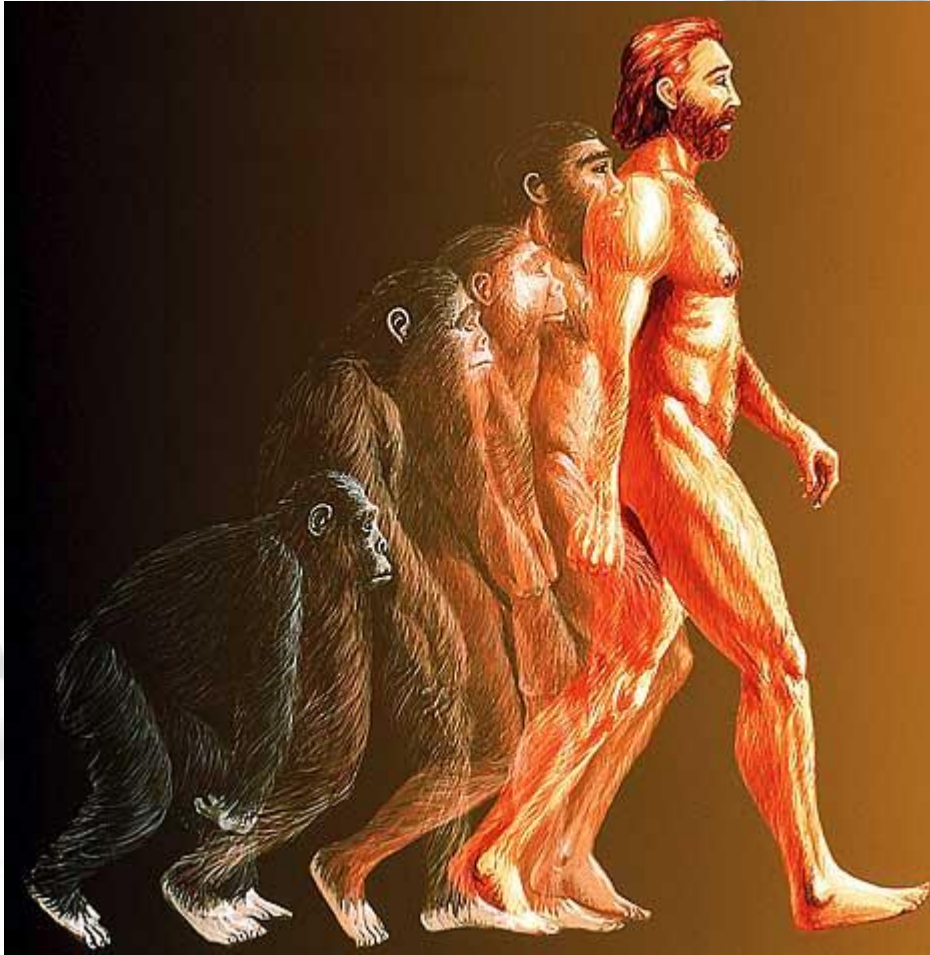




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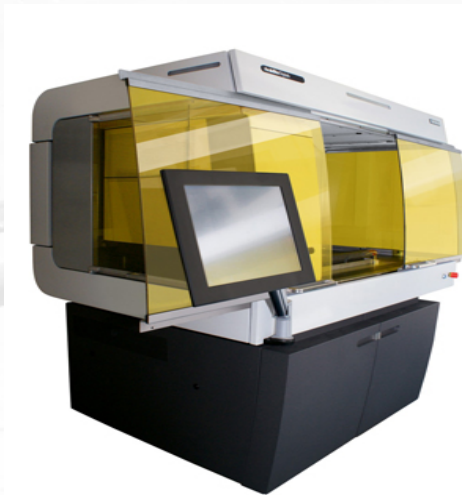
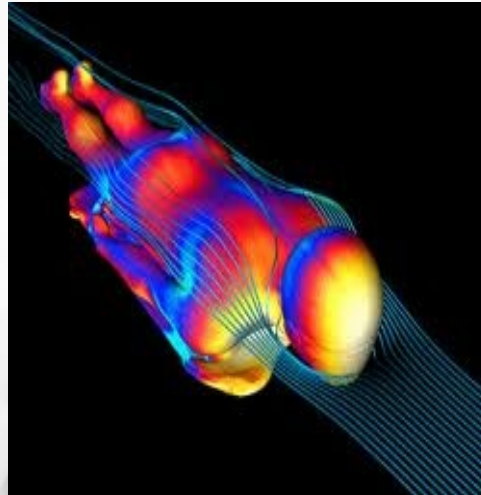


# Time motion of athlete's life



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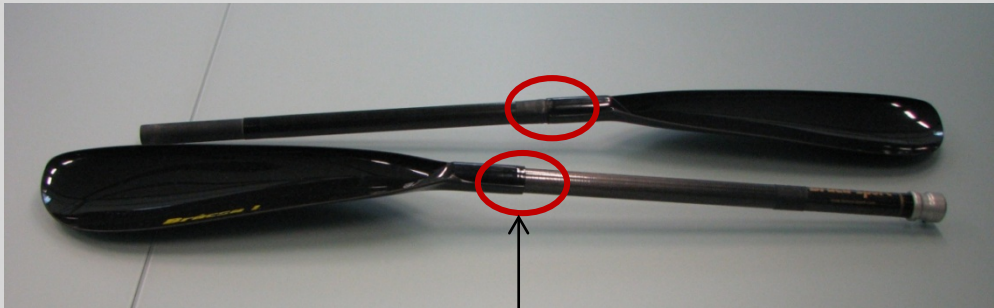
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## Swimming Race Simulator used to model a 100 m freestyle race

- Current simulation based on Subject G, time: 68.40s
- 2.07% reduction in underwater resistance (underwater phases), time: 68.09s
- 0.86% reduction in surface active resistance (surface phases), time: 68.24s
- Combined 2.07% reduction in underwater resistance and 0.86% reduction in surface active resistance, time: 67.89s

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Force measurement



Athlete and boat monitoring



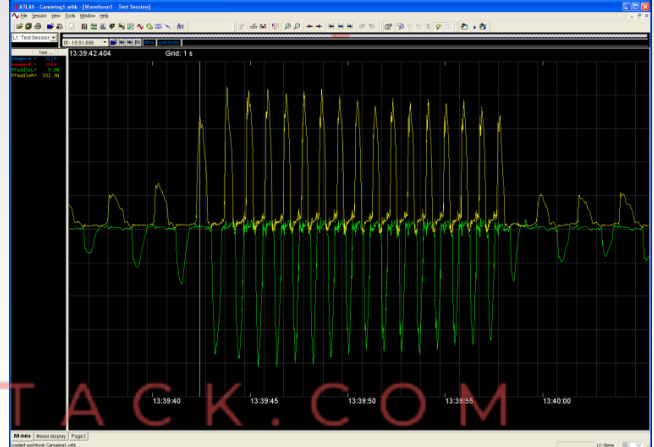
Receiver/transmitter inside shaft



Instrumentation

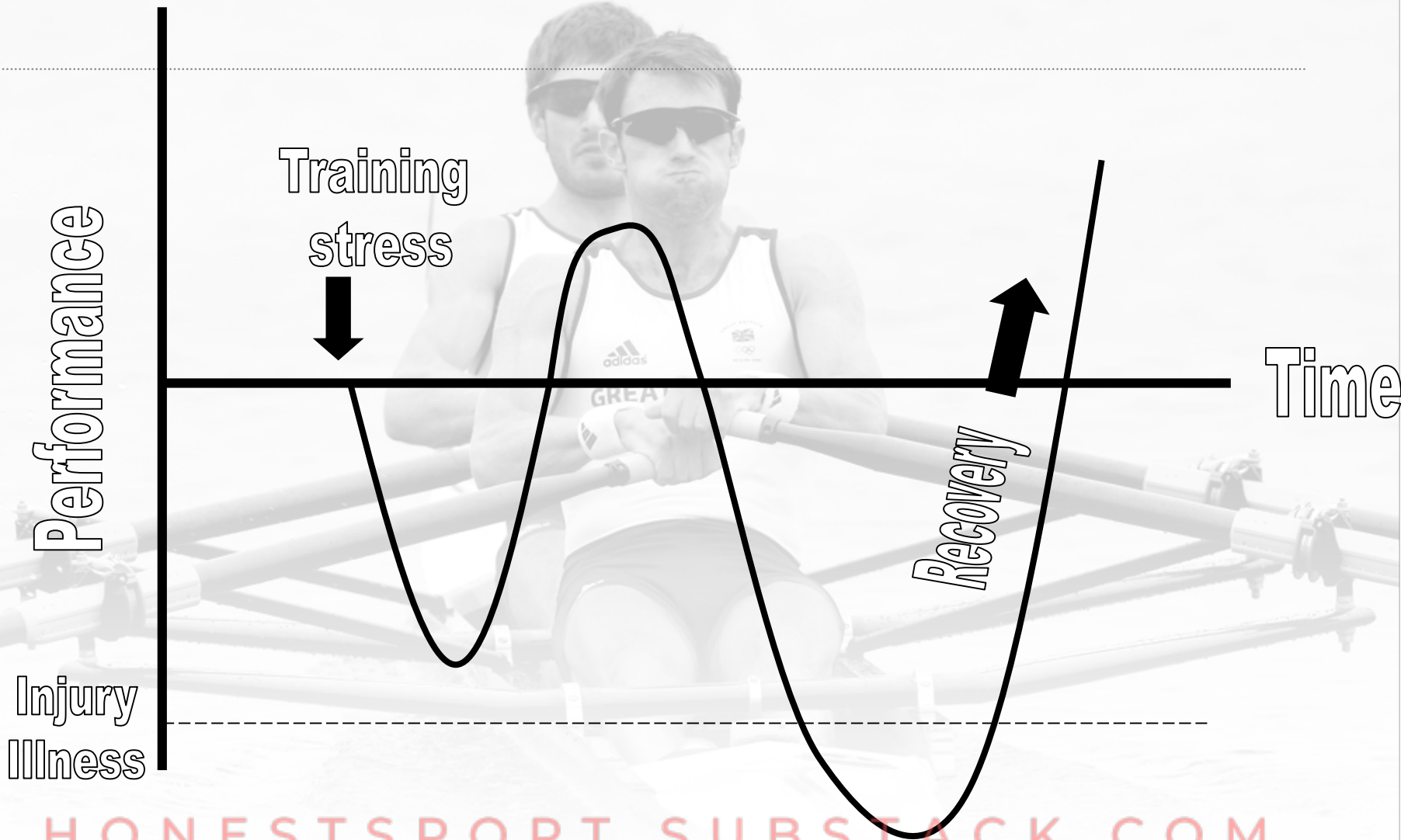


Wireless integration



Performance data analysis

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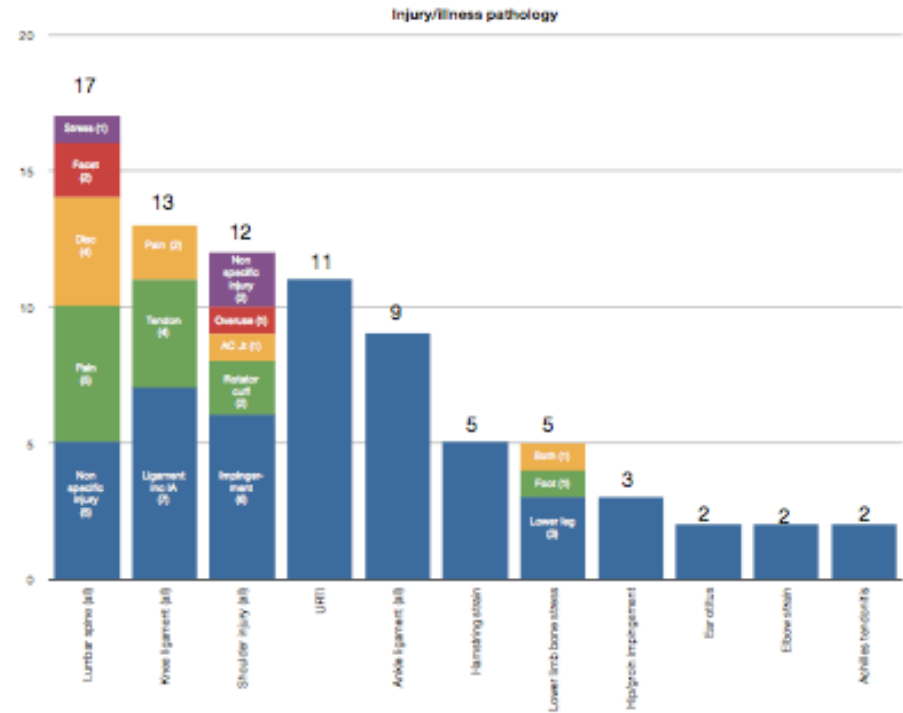
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# Injury & illness in Great Britain Sport

Olympiad review August 2009



Figure 3







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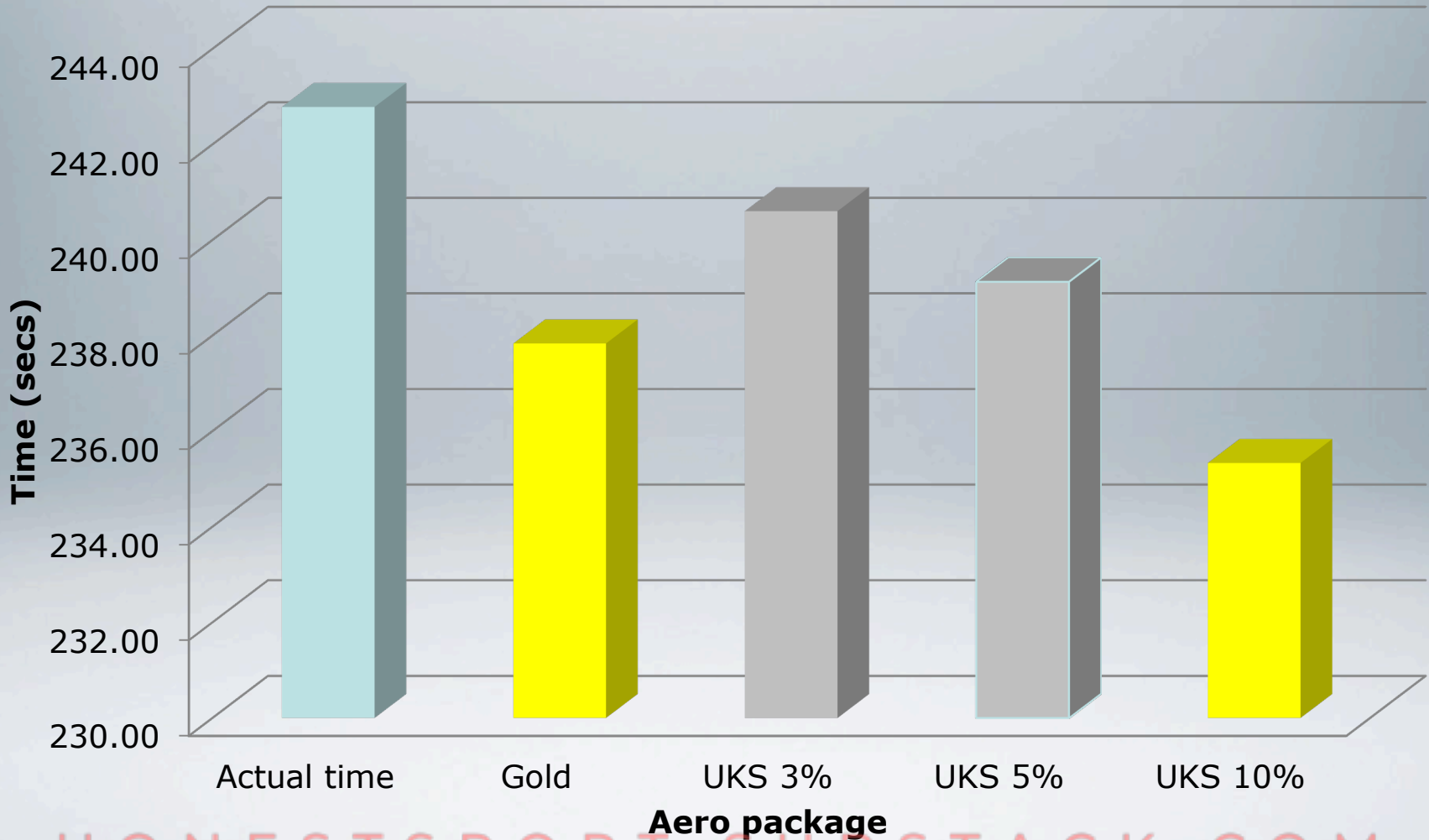


Does it matter?

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## Team Pursuit - Men



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# Innovation Landscape

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H O N E S T S P O R T . S U B S T A C K . C O M

# How to be a Civil Servant

second edition



Martin Stanley



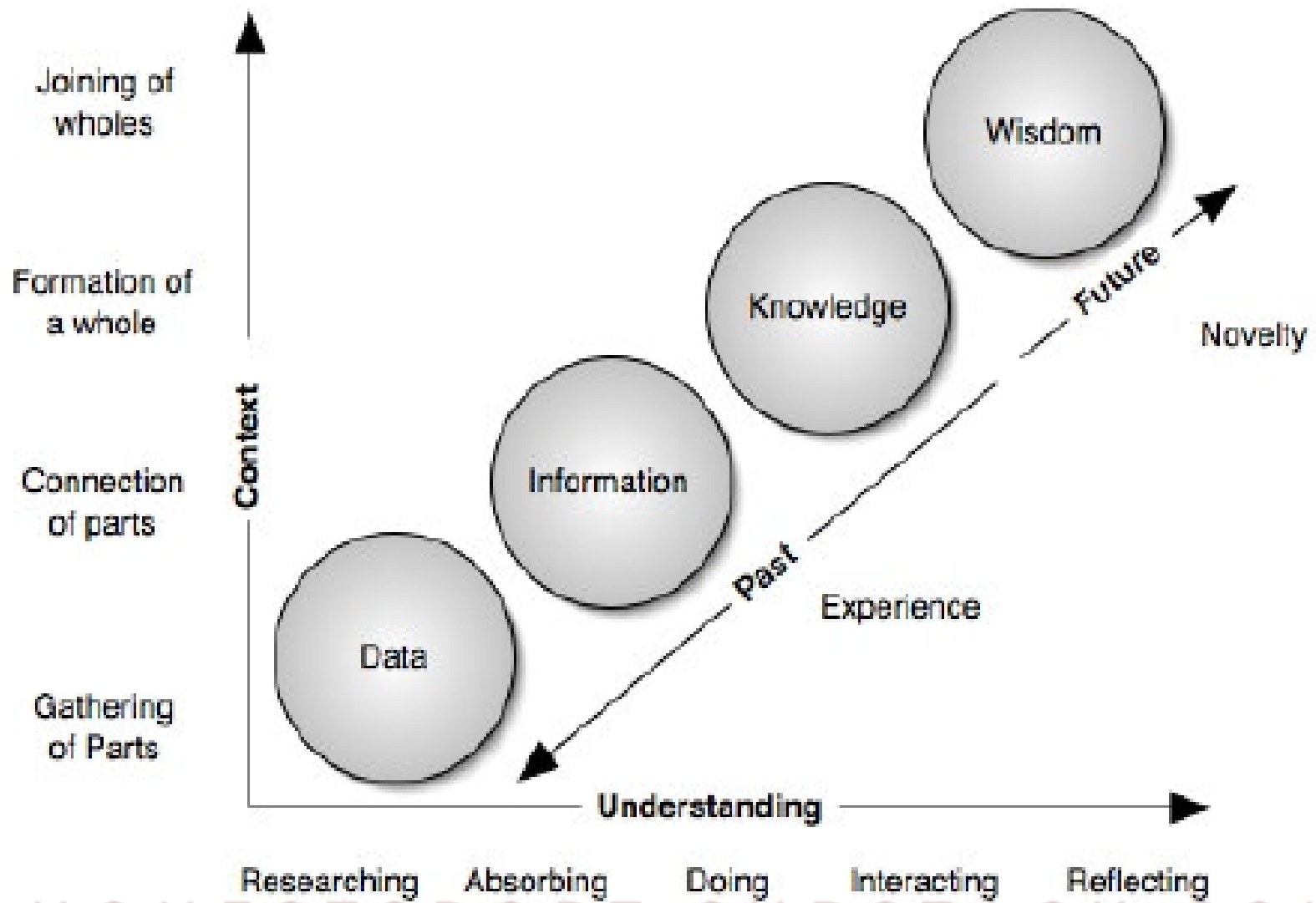
## managing research, development, and innovation

THIRD EDITION

Managing the Unmanageable

RAVI K. JAIN • HARRY C. TRIANDIS  
CYNTHIA W. WEICK

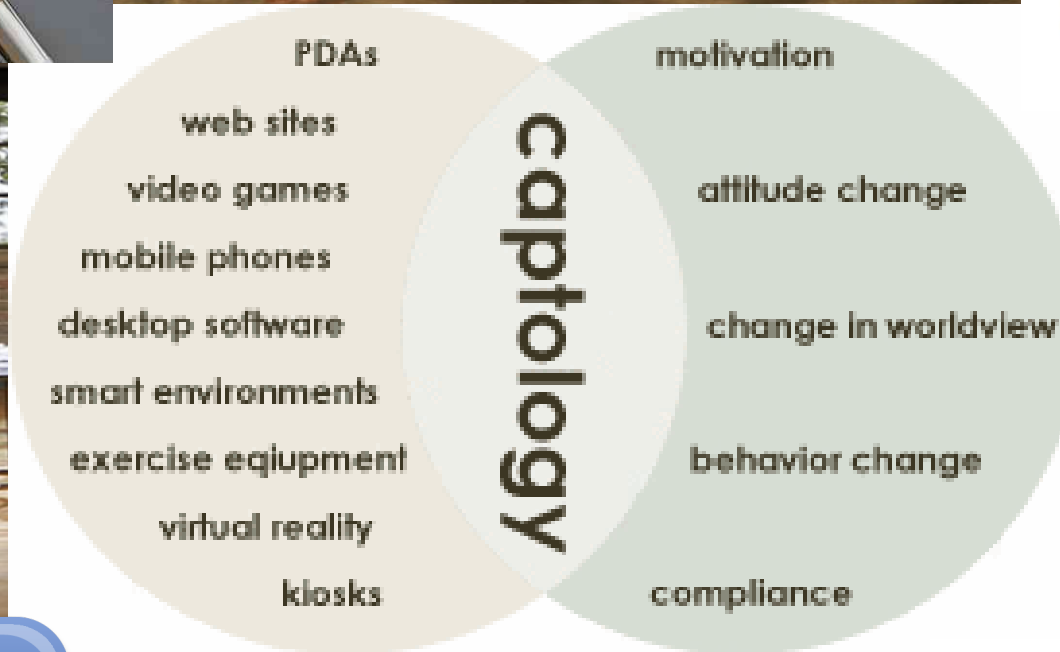
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twitter



computers

persuasion



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A background image of a cyclist in a white Great Britain jersey and helmet, celebrating with arms raised. The image is overlaid with a semi-transparent blue filter.

# One to consider – a novel breakthrough

H O N E S T S P O R T . S U B S T A C K . C O M



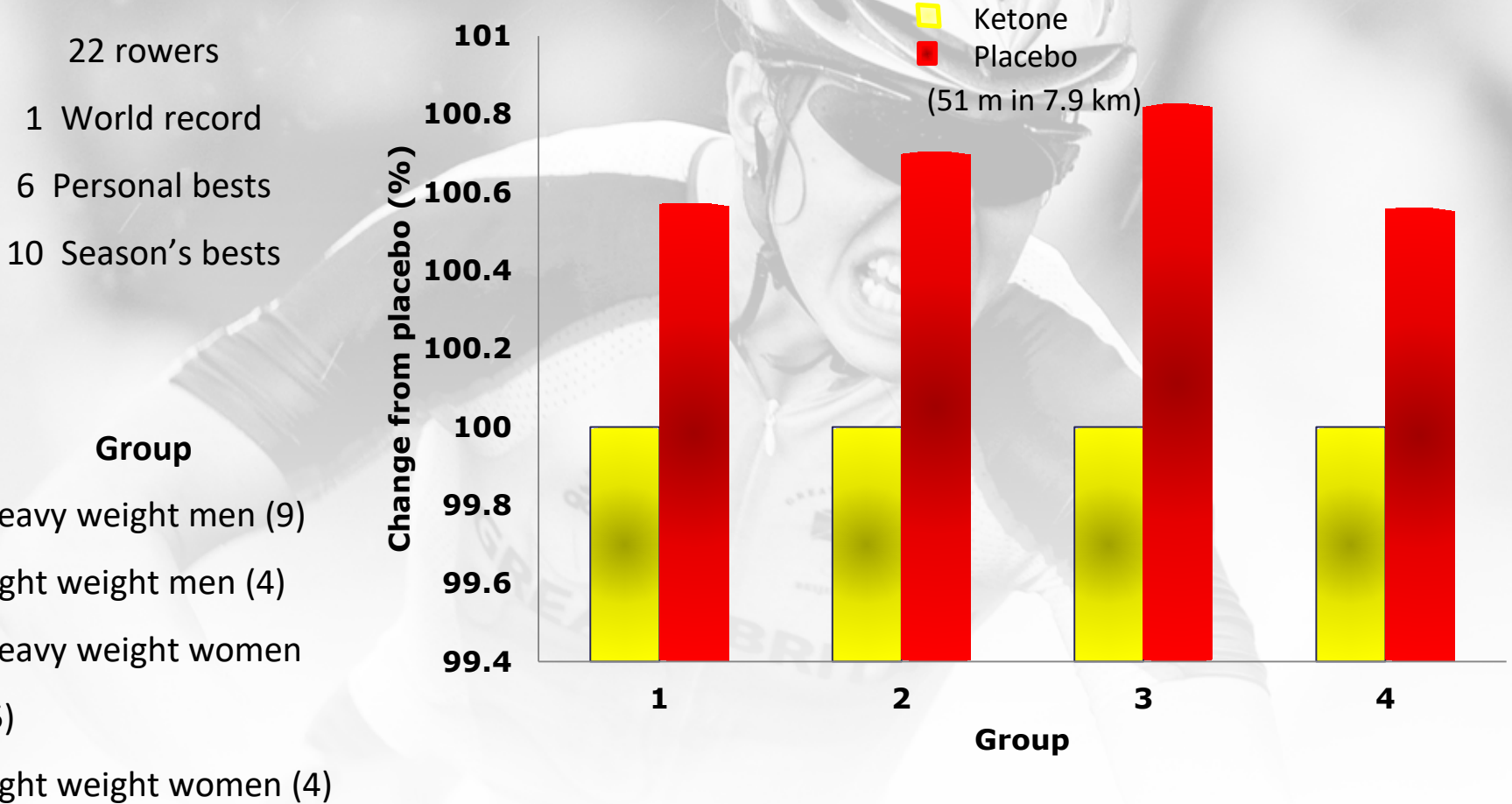
# A unique performance opportunity

- In 2007, UK Sport R&I team picked up on a short article - 'Superhuman' in the New Scientist (25 July, 2007, issue 2614).
- The article outlined some background work on a new nutritional fuel source (DeltaG) which had been developed with US military in partnership with Oxford University (\$10M funded).
- After 3 years of animal and human safety, toxicity, FDA approvals, UKS re-engaged and helped Oxford Uni construct and run a human performance trial.



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# British elite rowers – 30 minute distances

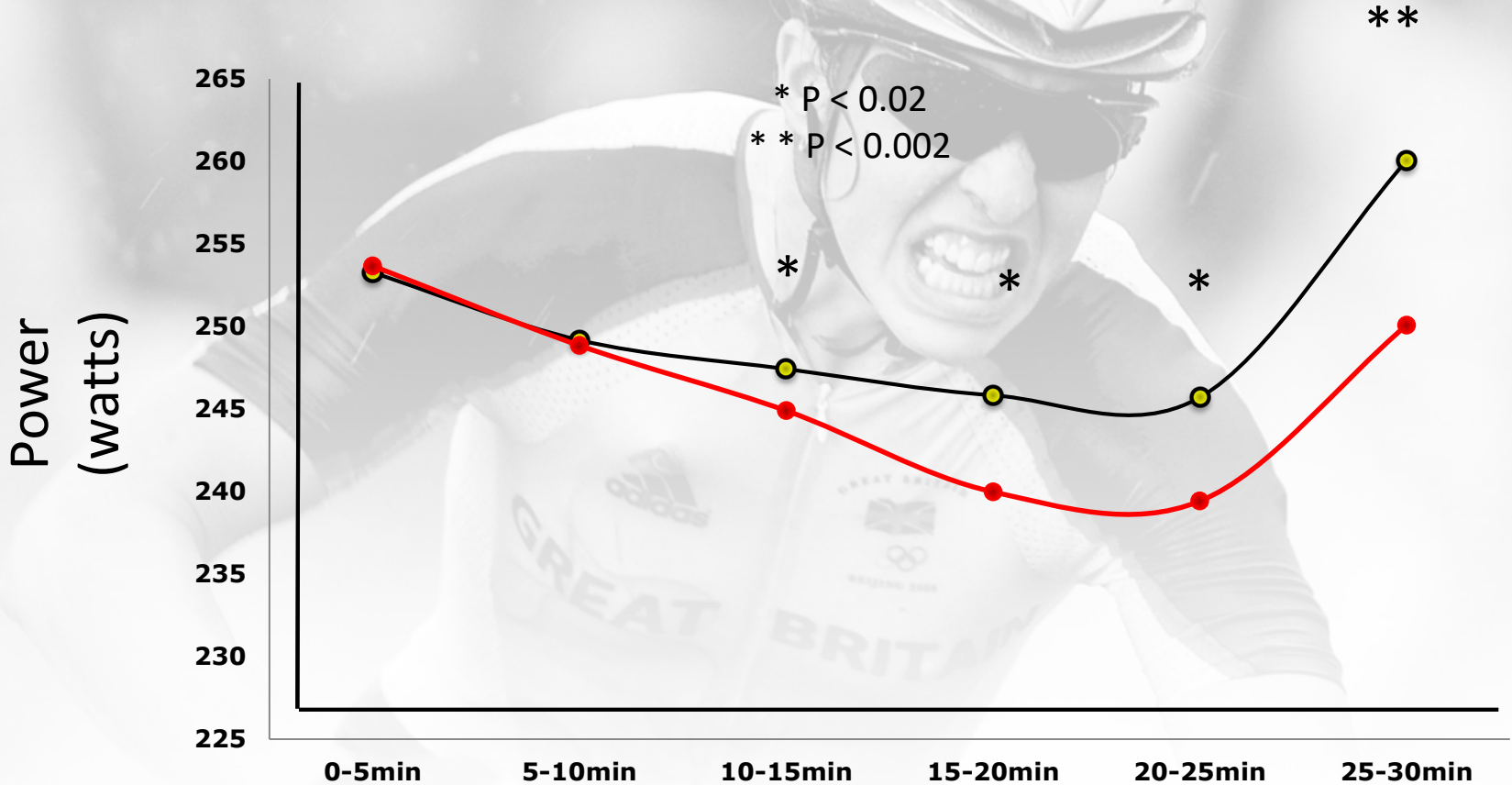


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TDS  
delta

# British elite rowers – Power over 30 min



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## Next steps

- Development of sufficient performance evidence for acute use during a single competitive tournament;
- UKAD approval and HFL quality assurance of the product manufacture line;
- An effective agreed communication strategy;
- Resourcing from within the UKS Performance budgets (for research phase);
- Commitment from GB sports to final costs (£50 / dose);
- An approved ethics proposal supporting the work at the Olympic and Paralympic Games.

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## Project Application Form

### 1. General

#### 1.1 Ref Number

RAG 01-12

#### 1.2 Project Title

The effects of acute supplementation with a ketone ester on training and competition performance in [REDACTED]: A repeated measures case study series

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]



## 1.8 Lay Summary (200 words maximum)

The human body produces ketones from stored fats in states of fuel shortage, to provide a more rapid and efficient alternative energy supply to active tissues, such as brain, heart and muscle. Recently, a novel ketone ester drink has been developed that can raise ketone levels in the body when consumed as part of a normal diet, even when other fuels are in plentiful supply. Circulating ketones, induced by consumption of the ester, are rapidly metabolised during exercise, with controlled trials in ■■■ athletes showing a positive impact on simulated sports performance both in ■■■ performing a 30 minute ■■■ test and in ■■■ performing an 80 minute ■■■ simulation.

In order to investigate the real-world impact of acute supplementation with the ketone ester drink, a series of case studies will examine repeated use of the ester during sports identified as having the potential to benefit from ketone consumption, based on current research findings and logical hypotheses based on current understanding of mechanisms of action. The effects across multiple sports over multiple performances will be examined by employing a repeated measures case study series.

## 1.9 Abstract

See Layman's summary

## 1.10 Project Aims and Hypotheses

The purpose of this investigation is to assess the effects of acute supplementation with a ketone ester on sports performance across a range of ■■■ through a series of case studies involving repeated interventions over multiple performances.

## 2. Project Overview (1000 words maximum)

The brain can use only two fuels, glucose and ketones. In states of fasting, when the supply of glucose decreases, the body breaks down fat to produce ketones (a state of 'ketosis'). Ketones are normally produced in the liver from dietary fat (high fat/low carbohydrate diets) or stored fat (during prolonged fasting) as a mechanism to supply energy for brain, heart and muscle function. Since ketones are produced as weak acids, prolonged excessive production can lead to complications, such as in diabetic ketoacidosis<sup>1</sup>.

It has been reported that ketones may be a more efficient energy source than glucose<sup>2</sup>. A diet inducing mild ketosis, via carbohydrate restriction (ketogenic diet), has been reported to improve abnormal movements, within days, in patients with Parkinson's disease<sup>3</sup> and to reduce the frequency of seizures in patients with epilepsy<sup>4</sup>.

A major issue with a ketogenic diet is that strict carbohydrate restriction (<20 g/day) must be enforced to raise circulating ketone levels. The high fat and low carbohydrate content make the diet unpleasant, with the result that ketogenic diets are poorly tolerated and have been linked to side effects such as increased plasma atherogenic lipoproteins, constipation, kidney stones and gallstones<sup>5,6</sup>.

Recently, an edible ketone, in the form of an ester, has been developed in an attempt to provide an alternative to ketogenic diets for Parkinson's disease and epilepsy<sup>7</sup>. It is now possible to raise circulating ketone levels, with no acid load, in a dose dependent fashion



by providing ketone esters in a drink, which can be consumed as part of a normal diet. This may offer the same benefits as the ketogenic diet, without the need for carbohydrate restriction. Since the ketone drink provides an alternative source of energy with potential benefits to metabolism, it is of great interest to investigate its role as an energy drink during exercise.

In previous unpublished research, we have shown that acute dietary supplementation with this novel ketone ester induces a sustained ketosis and improves endurance exercise performance in ██████████ performing a 30 min ██████████ time-trial and in well-trained ██████████ during an 80 min ██████████ simulation protocol. The ketone ester has been developed over 15 years of research at the University of Oxford, including concept development in rodents, safety and tolerability work in humans and ongoing laboratory based functional and mechanistic human trials. Further research is now required, to investigate the ergogenic potential of acute supplementation with this ketone ester during real-world performance conditions in ██████████ athletes.

The practice of inferring the real world value of interventions aimed at improving sports performance, such as nutritional supplementation, by examining their impact on laboratory performance measures or physiological surrogates, has attracted criticism<sup>8</sup>. Whilst surrogate performance measures observed in highly controlled settings improve internal validity and increase the likelihood of a clear outcome, their translation to the externally valid world of ██████████ performance is often questionable, particularly where a less highly trained population has been studied in an area where training status is likely to be a factor in the measured response. The difficulties inherent in gaining meaningful access to elite athletes has led some researchers to suggest that a more valid approach to establishing the efficacy of a sports performance intervention is to maximise the external validity of the research setting by examining athletes in their normal training or competition environment, whilst concurrently monitoring the many factors known to influence performance in this setting<sup>9</sup>.

The aim of the present study is to conduct a series of ██████████ athlete case studies, over several competition seasons, to describe the impact of repeated dietary supplementation with a ketone ester on sports performances both in training and in competition, including the ██████████ and ██████████. Repeated acute interventions with this novel nutritional intervention (NNI) will be characterised by conducting detailed performance analysis within each case study, with full consideration of the key factors known to impact performance outcome for a given sport. Athletes will also be asked to complete subjective ratings both of tolerance to the NNI and of perceived well-being whilst using the NNI during performance, through the use of affect rating scales validated for use in the sports nutrition setting<sup>10</sup>.

The study described in this generic protocol represents a unique opportunity to recruit participants from the short list of athletes likely to represent ██████████. These seasoned elite athletes have extensive performance histories, which have been diligently characterised by their scientific support staff in relation to the key factors contributing to performance outcome. These factors make it possible, through the methods described here, to conduct a minimally intrusive series of case study interventions, with the potential to explore the effects of an NNI whilst maintaining the ecological validity of observing ██████████ athletes in their natural habitat of sports training and competition. The outcomes of this descriptive study are likely to inform the direction for further experimental work with the NNI.





regime to ensure the delivery of a moderate sustained ketosis during performance level demand.

The starting point for development of optimal dosing will be ingestion of ketone ester at up to 8 mg/kg body mass/min of exercise, which has been effectively proved in previous trials for inducing a sustained moderate ketosis (blood betahydroxybutyrate: ~2-4mmol/l). The ketone ester will be delivered as a drink in shot form (~60-80ml per dose), against a background of carbohydrate (~1:1 ratio), flavours and sweeteners, which assists in improving the palatability of the drink. Water will be provided *ad libitum* and athletes will be free to supplement their carbohydrate intake with other sports drinks or food stuffs.

In some case studies, sports may choose to supplement the case study design with a placebo controlled, randomised cross-over intervention, to provide further evidence for the efficacy of the NNI. Where this is the case the details will be outlined in the annexe for that case study.

During the preliminary testing and some of the performance interventions, and by agreement with coach and athlete, capillary blood samples (less than 50 uL) will be taken (SAP R3) prior to supplementation, pre-exercise, during exercise and post-exercise for the immediate analysis of blood ketone, lactate and glucose levels using portable test meters (Optimum Xceed, Abbott Diabetes Care, Berkshire, UK; LactatePro, Arkray Inc., Japan).

#### *Treatment with the NNI*

For each case study a number of repeat interventions (target range: 2-10 repeat trials) with the NNI will be negotiated with the coach and athlete(s) to be included in performances both in competition and competition simulation in training. Athletes continuing to compete at the international level after the [REDACTED] will be invited to continue with the study by engaging in further performance interventions with the NNI up to and including the [REDACTED]. Dosing regimes for all case study interventions will follow the guidelines established in the preparatory work for each case study.

All drinks will be specifically formulated by the project team, produced and bottled (in sealed bottles) by an independent food specialist ([www.intlfoodnetwork.com](http://www.intlfoodnetwork.com)) and batch-tested ([http://www.lgc.co.uk/divisions/hfl/hfl\\_sport\\_science.aspx](http://www.lgc.co.uk/divisions/hfl/hfl_sport_science.aspx)) before delivery to the location of the study to minimize the risk of contamination of the product with substances which contravene the World Anti-Doping Agency (WADA) code.

#### *Data Collection*

Over the course of an 8-32 month period, all international competition performances and selected competition simulations in training (both with and without the NNI) will be characterised and thereby considered for inclusion in each case study. Depending on the components of each case study, co-factors will be recorded (e.g. home advantage; technicality of course/opposition; concurrent nutritional and/or technical interventions) alongside performance analysis and outcomes, and may be complemented with historical performance data where available.

### **3.4 Data Analysis**

Data from each case study will be described in relation to the apparent magnitude of effect of the intervention, both on actual outcome and perceived benefit, its repeatability over multiple uses and its relationship to the factors identified as impactful to performance

outcome for each sport. If the impact of the NNI appears robust and repeatable across the case studies the data may be normalised and pooled for statistical analysis.

### **3.5 Reporting & Implementation**

No access or dissemination of personal information will be allowed without formal written consent of the participant. No data collected in this study will affect athlete selection for competitions or teams. All data collected are strictly confidential. No results will be disseminated to parties other than the investigation team, coach or athlete. A brief report will be provided to UK Sport. Anonymised results will likely be prepared for presentation at sporting and scientific conferences and for publication in a relevant academic journal.

## **4. MEDICAL AND ETHICAL CONSIDERATIONS**

This study complies, and at all times will comply, with the Declaration of Helsinki, as adopted at the 52<sup>nd</sup> WMA General Assembly, Edinburgh, October 2000 (Details given at <http://www.wma.net/e/policy/b3.htm>) and the Data Protection Act 1998.

This application utilises procedures that fall within previously approved Schedules of Approved Procedures (SAP) in a recognized/accepted protocol: A5, B1, H1 & H2, I3 & 5, K2, and R3 from RAG SAP.

“Atkins” style diets are well known to the general public and their effectiveness is mediated around the generation of ketones for use as fuel rather than carbohydrates with no known risks to health. The ketone ester used in this study is not commercially available and has been designated as GRAS (Generally Recognised As Safe) for use in research by the FDA (Food and Drug Administration) in the USA (report attached). A further report by [REDACTED] to the world anti drug-doping association (WADA) provides details regarding toxicology studies pertaining to the safety of the ketone ester, in addition to information on the absorption, distribution, metabolism and elimination of this ester (see attached). On reviewing the attached dossier, the response from WADA was: “At this point in time and based on the confidential information from TAS we have reviewed, we have no reason to consider such substances as banned under the 2011 List of Prohibited Substances and Methods.”

Previous studies using this supplement have not been considered to be clinical trials of an investigational medicinal product by the Medicines and Healthcare products Regulatory Agency (MHRA), as defined by the EU directive 2001/20/EC (e-mail correspondence letters from the MHRA attached); LREC reference numbers O7/Q1605/37 and 10/H0605/10. The ketone ester has also been used by our group in placebo controlled performance trials with [REDACTED] under the approval of the institutional ethics committee at the University of Bath. For reference, feedback from an independent scientific review regarding the safety and potential directions for future research is also attached.

Interventional drinks will be screened for the presence of a wide range of substances prohibited by WADA at an (ISO 17025) accredited sports testing laboratory (HFL, Cambridge).

The ketone ester has been tested on [REDACTED] (?) volunteers. It is bitter in taste and is not particularly palatable. However, it is generally well tolerated, with occasional reports (in an estimated 20% of cases) of mild gastro-intestinal upset (e.g. mild bloating, abdominal



pressure and belching) for a short period following consumption, and 5 reported cases from previous research of nausea and vomiting, which spontaneously resolved upon regurgitation of the drink. There have been no reported cases so far of GI upset resulting in premature termination of exercise.

The withdrawal criteria for this study will be:

- a. On the decision of a participant
- b. On the decision of the PI
- c. On the decision of the IMO
- d. On the report by a participant of any significant (greater than “mild”) adverse reaction (including GI disturbances)

Participants will be closely monitored for adverse reaction using formalised rating scales and will be removed from the study if they demonstrate more than a mild adverse reaction (e.g. GI disturbance). The occurrence of any such adverse reaction will be recorded and reported back to the RAG.

Written, informed consent will be obtained from all participants before they commence the study. Participants can withdraw their consent at any time throughout the course of the project and without requirement to provide a reason.

## **5. HAZARDS TO PARTICIPANT SAFETY**

High-intensity exercise carries a small risk to participants, but all participants will be [REDACTED] and this risk is therefore extremely small, while participants will be performing the type of exercise that is a normal part of their everyday training and competition programme.

The ketone ester has been tested on [REDACTED]. It is bitter in taste and is not particularly palatable. However, it is generally well tolerated, with occasional reports (in an estimated 20% of cases) of mild gastro-intestinal upset (e.g. mild bloating, abdominal pressure and belching) for a short period following consumption, and 5 reported cases from previous research of nausea and vomiting, which spontaneously resolves upon regurgitation of the drink. There have been no reported cases so far of GI upset resulting in premature termination of exercise.

Any type of nutritional intervention holds a general risk of consuming illegal or contaminated substances. However, the ketone ester is currently not on the banned list of substances by WADA (World Anti-doping Agency); and intervention as well as placebo drinks are screened for the presence of a wide range of substances prohibited by WADA at an (ISO 17025) accredited sports testing laboratory (HFL, Cambridge).

Capillary blood samples will be taken by a researcher appropriately trained in blood sampling. Blood sampling may cause very minor bruising, but good practice minimizes the risk, and the taking of blood samples is a common practice in elite sport. Participants are free to choose not to agree to blood sampling, either at the outset or at any point during the process, without prejudice to their wider participation in the study.

If it becomes apparent during the course of the study that this assessment of risks changes in any way, UK Sport, the RAG and those participating in the study will be informed fully and without delay. Similarly, UK Sport and the RAG will be informed without delay in the

event that unforeseen adverse events arise in the course of this research. The latter report shall contain an evaluation of the cause and severity of the adverse event.

## 6. REFERENCES

1. Kitabchi AE, Umpierrez GE, Murphy MB, Kreisberg R a. Hyperglycemic crises in adult patients with diabetes: a consensus statement from the American Diabetes Association. *Diabetes care*. 2006;29(12):2739-48.
2. Veech RL, Chance B, Kashiwaya Y, Lardy HA, Cahill GF. Ketone bodies, potential therapeutic uses. *IUBMB life*. 2001;51(4):241-7.
3. Vanitallie TB, Nonas C, Rocco a Di, et al. Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study. *Neurology*. 2005;64(4):728-30.
4. Kossoff EH. More fat and fewer seizures: dietary therapies for epilepsy. *Lancet neurology*. 2004;3(7):415-20.
5. Kwiterovich PO, Vining EPG, Pyzik P, Skolasky R, Freeman JM. Effect of a high-fat ketogenic diet on plasma levels of lipids, lipoproteins, and apolipoproteins in children. *JAMA : the journal of the American Medical Association*. 2003;290(7):912-20.
6. McNally M a, Pyzik PL, Rubenstein JE, Hamdy RF, Kossoff EH. Empiric use of potassium citrate reduces kidney-stone incidence with the ketogenic diet. *Pediatrics*. 2009;124(2):e300-4.
7. Kashiwaya Y, Pawlosky R, Markis W, et al. A ketone ester diet increases brain malonyl-CoA and Uncoupling proteins 4 and 5 while decreasing food intake in the normal Wistar Rat. *The Journal of biological chemistry*. 2010;285(34):25950-6.
8. Atkinson G, Nevill A. Selected issues in the design and analysis of sport performance research. *Journal of sports sciences*. 2001;19(10):811-27.
9. Smith TB, Hopkins WG. Variability and predictability of finals times of elite rowers. *Medicine and science in sports and exercise*. 2011;43(11):2155-60.
10. Backhouse SH, Biddle SJH, Bishop NC, Williams C. Caffeine ingestion, affect and perceived exertion during prolonged cycling. *Appetite*. 2011;57(1):247-52.

## Participant Information Sheet

You are invited to take part in a research study. Before you decide 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 us 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.

Also, please consider that you have signed a non disclosure agreement. This means that you cannot talk to anybody outside of the research team and your peer group, i.e. athletes and coaches who have also signed this NDA, about the details of this study, especially not about the type of nutritional intervention you are testing.

### Study title

The effects of acute supplementation with a ketone ester on training and competition performance in [REDACTED]: A repeated measures case study series

### What is the purpose of the study?

This study aims to investigate the effects of a drink containing a ketone ester on sports performance in training and competition across a number of [REDACTED].

### Background information

In states of fuel shortage, like fasting, the body converts fat into ketones in order to meet the energy needs of the brain, which is unable to utilise fat when carbohydrate levels are low. Ketones can also provide a rapid and efficient alternative to carbohydrate or fat for energy supply to heart and muscle, making them of therapeutic interest as an alternative fuel source. It is possible to raise ketone levels in the body by restricting carbohydrate intake and consuming a diet high in fat. However, this type of diet is difficult to tolerate for most people.

Recently, an edible form of ketone has been developed that can be consumed in a drink as part of a normal diet. Since the ketone drink provides an alternative energy source, we are investigating its role as an 'energy drink' during exercise. Initial trials have shown a positive impact on exercise performance in elite [REDACTED] performing a [REDACTED] and well-trained [REDACTED] during an 80-min [REDACTED] simulation protocol. Further research, such as this study, is needed to investigate the impact of this ketone ester during real-world sports performance in training and competition.

### Invitation to participate

We invite you to participate in a research project examining the effect of acute ingestion of a ketone ester drink on sports performance.



## Do you have to take part?

Participation in this study is completely voluntary and 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 asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason. If you decide not to take part or to withdraw from the study, this will not affect your future training or athlete status in any way. Likewise, you may withdraw from the study at any time, or may be withdrawn on request of the independent medical officer, but will suffer no penalty as a result.

## Procedures

Should you agree to participate in this study then you will be asked to continue with your normal training and competition programme. A time for you to participate in tests will be arranged with yourself and your coach by mutual agreement.

Firstly, we will ask you to participate in preliminary sessions to familiarise you to the test drink and to ensure that you find the drink tolerable (as it is quite strong and bitter in taste). This means, you will consume the test drink around your normal training sessions and we will ask you some basic questions about the taste of the drink and may take some simple capillary blood samples from an earlobe or fingertip to determine the effects of the drink (see below).

Once you are sufficiently familiarised you will be invited to consume the test drink during performances conducted both in training and competition, on multiple occasions (target 2-10 trials) over an 8-32 month period. Your continued involvement across the study period is entirely at your own discretion and you are free to withdraw at any point without giving a reason. On each occasion you will be asked to consume a bolus of the test drink (typically ~150-250mL) around one hour before your performance and to top this up with small shots of the test drink (~60-80mL) at regular intervals during your performance, depending on its intensity and duration and the practicalities for your sport.

We will record the results of these performances alongside other performances when the test drink is not being used, and will record a number of other factors identified by your support team as being influential to your performance outcome, in order to determine the overall effects of consuming the ketone ester. We will also ask you to complete subjective rating scales evaluating your tolerance of the drink as well as your overall feeling of well-being whilst performing with the drink. Your data will be anonymised before being compared to the performances of other people in your sport and to other athletes taking part in this study from other sports.

All test drinks are specifically formulated by the project team and we can provide you with a general breakdown of the ingredients if this is required. The drinks you will be provided with are produced and then bottled in sealed containers by an independent food specialist and batch-tested for contamination with substances which contravene the WADA code.

On some occasions, and upon agreement with yourself and your coach, capillary blood samples will be taken prior to supplementation, pre-exercise, during exercise and post-exercise for the analysis of blood ketone, lactate and glucose levels using portable test meters. A trained researcher will apply a small pin prick to either your fingertip or earlobe to collect a very small amount of blood (less than 50  $\mu$ L) onto a test strip.

## Requirements

- You will be required to partake in testing sessions on 4-12 occasions.
- Your total time commitment for this study will range between 8-48 hours spread across an 8-32 month period.
- Any other nutritional supplements that you consume during the testing period should be documented and wherever possible repeated across trials.
- You must not be pregnant or have the possibility of becoming pregnant during the trial period.

## What are the benefits of taking part?

The possible benefits of dietary strategies to maximize exercise performance are still unclear and the information obtained from this study will provide useful information to help in understanding factors that influence performance and help to plan future methods to maximise your performance. There are no other benefits of taking part. You can withdraw from the experiment at any time without giving a reason.

Consumption of the ketone ester may improve your [REDACTED] performance. However, it is also possible that your performance could be adversely affected. For example, the unpleasant taste of the drink may be detrimental to your performance in some way. Your decision whether or not to use the ketone ester in a [REDACTED] is entirely your own and should be taken in full knowledge of the risks outlined here.

## What are the risks of taking part?

- High-intensity exercise carries a small risk, but you will be performing the type of exercise that is a normal part of your everyday training and the risk is no greater with or without the ketone ester.
- Capillary blood samples will be taken by a researcher appropriately trained in blood sampling. Blood sampling may cause very minor bruising, but good practice minimizes the risk. Should you choose not to agree to blood sampling, either at the outset or at any point during the process, you can still take part in all other aspects of the study.
- The ketone ester used in this study is not commercially available and can only be administered as part of a research trial. It has been previously tested on over 80 healthy male volunteers. It is bitter in taste and is not particularly palatable. However, it is generally well tolerated, with occasional reports (in an estimated 20% of cases) of mild gastro-intestinal upset (e.g. mild bloating, abdominal pressure and belching) for a short period following consumption, and 5 reported cases from previous research of nausea and vomiting, which spontaneously resolved upon regurgitation of the drink. There have been no reported cases so far of GI upset resulting in premature termination of exercise. If you do not like the taste of the drink and experience any gastrointestinal discomfort you are free to withdraw at any time.

Any use of a nutritional supplement holds a risk of consuming illegal or contaminated substances. However, after discussions with UKAD (UK Anti Doping) and WADA (World Anti Drug-Doping Agency) in September 2011, we can confirm that the ketone ester used in this study is currently not on the banned list of substances by WADA; and the ketone drinks, as well as their production lines, are tested by HFL Sport Science Ltd for a wide range of substances banned by WADA (however, it will not test for all substances), a World Class sports doping control and research laboratory, with more than 47 years of leadership in drug surveillance in sport.

Please note that the confirmation above does not represent a formal waiver by UKAD or

WADA of the use of ketone esters for this study or otherwise and as such its use still remains regulated by the World Anti-Doping Code. UK Sport does not guarantee, promise, assure or represent that the ketone ester is absolutely World Anti-Doping Code compliant and therefore excludes all responsibility for the use of ketone ester.

### **What will happen to the results of this study?**

The results of the research will be available within 3 months of all the data being collected. All results will remain confidential. The data we collect will be stored on password protected laptops by project team members. All personal information will be removed prior to any subsequent publication. You can only receive your own results and not those of others.

### **Who has reviewed this study?**

This study has been reviewed by the UK Sport Research Advisory Group ethics committee.

### **Who is funding this research?**

This study is being funded by the UK Sports Council.

### **Many thanks for considering participation in the study**

If you have any further queries, please do not hesitate to contact us. If you agree to take part in this study then you will be given a copy of the Information Sheet and a signed Consent Form to keep.

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]



## Participant Consent Form

### **The effects of acute supplementation with a ketone ester on training and competition performance in [REDACTED]: A repeated measures case study series**

1. I have read the information sheet, which provides full details of this study, and have had the opportunity to raise and discuss my questions with a named researcher regarding the general nature, object, potential risks and duration of the study, and understand what is expected of me.
2. I understand that the aim of the study is to investigate the effects of a specifically formulated ketone ester drink on [REDACTED] performance.
3. I agree to volunteer as a participant for the study described in the information sheet. I give my full consent to my participation in this study.
4. I confirm that I do not know of any medical condition that I suffer from which might have the effect of making it more likely that I be involved in an incident which could result in injury to myself or others. I also confirm that I am not pregnant and there is no possibility of me being pregnant during the study.
5. As part of my agreement to participate I accept that there are risks in this study due to performing maximal exercise and to ingesting the ketone ester supplement.
6. I acknowledge that whilst efforts have been made to conduct checks (including checks with UKAD and WADA) on the use of ketone ester no promise, guarantee, representation or assurance can be made on its compliance with the World Anti-Doping Code or otherwise and UK Sport excludes all liability from any breach arising from the use of ketone esters under the World Anti-Doping Code or otherwise.
7. I consent to UK Sport and the project team members processing my data under the Data Protection Act 1998 for the purpose of this study only and for no other purpose whatsoever. I consent that UK Sport and the project team members may share my data unanonymised with my sport's World Class Performance Programme (NGB WCPP), for the purposes of assessing my performance under the study. Any other presentation of my own individual data outside of my NGB WCPP will maintain my anonymity unless I specifically give my permission otherwise.
8. I reserve the right to withdraw from this experiment at any time without giving a reason; I understand that I may be withdrawn at any time on request of the independent medical officer, and will suffer no penalty as a result.

Name of participant

Date

Signature

Name of investigator

Date

Signature

**Project Application Form**

**Annex 1**

**Ref Number**

RAG 01-12a

**Project Title**

The effects of acute supplementation with a ketone ester on training and competition performance in [REDACTED]: A repeated measures case study series

[REDACTED]

[REDACTED]

**Sport**

[REDACTED]

[REDACTED]

[REDACTED]

**Sport Specific Methods**

During all case study interventions a designated member of the personnel named below will be present and responsible for administering the study.

[REDACTED] will employ a proprietary video-based race analysis system which allows for the measurement of performance critical factors such as [REDACTED]

[REDACTED] both in training and in competition.

For each measured performance a number of co-factors will be recorded (to include: level of competition, training status, time of day, concurrent technical/nutritional interventions).

[REDACTED]

[REDACTED]



**Project Application Form**

**Annex 2**

**Ref Number**

RAG 01-12b

**Project Title**

The effects of acute supplementation with a ketone ester on training and competition performance in [REDACTED] sports: A repeated measures case study series

[REDACTED]

[REDACTED]

**Sport**

[REDACTED]

[REDACTED]

[REDACTED]

**Sport Specific Methods**

During all case study interventions a designated member of the personnel named below will be present and responsible for administering the study.

[REDACTED] will employ a [REDACTED] system (SRM) during both training and [REDACTED] performances, which allows measurement and recording of [REDACTED]

[REDACTED] For each measured performance a number of co-factors will be recorded (to include: [REDACTED] level of competition, training status, time of day, concurrent technical/nutritional interventions).

[REDACTED]





## Project Application Form

### Annex 3

**Ref Number**

RAG 01-12c

**Project Title**

The effects of acute supplementation with a ketone ester on training and competition performance in [redacted] sports: A repeated measures case study series

[redacted]

[redacted]

**Sport**

[redacted]

[redacted]

[redacted]

**Sport Specific Methods**

During all case study interventions a designated member of the personnel named below will be present and responsible for administering the study.

[redacted] will employ a [redacted] system [redacted] with proprietary data capture and analysis system) during both training and competition performances, which allows measurement and recording of [redacted] alongside measurement of segment

times and [REDACTED] via video analysis. For each measured performance a number of co-factors will be recorded (to include: [REDACTED] level of competition, training status, time of day, concurrent technical/nutritional interventions).

[REDACTED]

**Project Application Form**

**Annex 4**

**Ref Number**

RAG 01-12d

**Project Title**

The effects of acute supplementation with a ketone ester on training and competition performance in [REDACTED] sports: A repeated measures case study series

[REDACTED]

[REDACTED]

**Sport**

[REDACTED]

[REDACTED]

[REDACTED]

**Sport Specific Methods**

During all case study interventions a designated member of the personnel named below will be present and responsible for administering the study.

[REDACTED] will employ a [REDACTED] system [REDACTED] with proprietary data capture and analysis system) during both training and competition performances, which allows measurement and recording of [REDACTED] alongside measurement of [REDACTED] via video analysis and/or hand timing. For each measured performance a number of co-factors will be recorded (to include: [REDACTED] level of competition, training status, time of day, concurrent technical/nutritional interventions).

[REDACTED]





University of Bath



PHD

**The Power of Substrate: An Examination of the Physiological Basis for and Functional Impact of a Novel Nutritional Intervention for Sports Performance**

Smith, Al

*Award date:*  
2017

*Awarding institution:*  
University of Bath

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**The Power of Substrate: An Examination of the Physiological Basis for  
and Functional Impact of a Novel Nutritional Intervention for Sports  
Performance**

**Al Smith**

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department for Health

July 2016

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Signed on behalf of the Department for Health

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## List of Papers

*Authored publications directly resulting from work contributing to this thesis:*

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Peacock O, Tjonna AE, James P, Wisloff U, Welde B, Bohlke N, Smith A, Stokes K, Cook C, Sandbakk O. Dietary nitrate does not enhance running performance in elite cross-country skiers. *Med Sci Sports Exerc.* 2012;44(11):2213-2219.

Toone RJ, Peacock OJ, Smith A, Thompson D, Drawer S, Cook C, Stokes KA. Measurement of steroid hormones in saliva: effects of sample storage condition. *Scand J Clin Lab Invest.* 2013;73(8):615-621.

Cox, P., Kirk, T., Ashmore, T., Stubbs, B., Evans, R., Smith, A., ... Clarke, K. Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell Metabolism.* 2016;24(2):256-268.

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## Abstract

A novel form of nutritional ketosis has recently emerged, based on the consumption of a ketone ester, that has the potential to impact upon sports performance. Ketone bodies are not normally present in the diet in more than trace amounts and are only produced in significant quantities physiologically during times of extreme energetic stress, but appear to be a highly efficient fuel source. The presence of elevated levels of ketone bodies in the absence of calorie deficit is highly novel and represents a fascinating metabolic state both for the examination of substrate selection during exercise and for the potential to impact sports performance.

This PhD programme examined the utility of nutritional ketosis in sport by exploring the evidence base for its impact upon exercise performance and examining the translation of this knowledge into interventions in the field during real world sports performance. Study 1 (Chapter 3) established guidelines for dosing by demonstrating that consuming a meal in close proximity to ketone ester dosing (within 30-minutes (F30) compared to 90-minutes (F90)) resulted in a lowering of the area under the curve for ketone body concentration in the 90-minutes post dose (AUC-BHB(0-90), F90 v F30:  $260.7 \pm 33.6$  mmol/l°min v  $201.9 \pm 33.4$  mmol/l°min;  $P < 0.05$ ) and that reducing the carbohydrate content of the ketone ester drink (F4:1) resulted in an increased accumulation of circulating ketone bodies (AUC-BHB(0-90), F90 v F4:1:  $260.7 \pm 33.6$  mmol/l°min v  $311.4 \pm 49.3$  mmol/l°min;  $P < 0.05$ ). Study 2 (Chapter 4) showed that low-dose ketone ester consumption had no effect on long duration cycle performance (20km TT, KET v CHO:  $1731.3 \pm 113.0$ s v  $1736.9 \pm 114.2$ s;  $P = \text{N.S.}$ ) in contrast to prior evidence for performance impact at higher doses, suggesting that



there may be a threshold of nutritional ketosis required to confer ergogenic benefit. In study 3 (Chapter 5) the hormonal response to repeat sprint cycling performance was characterised with transient increases in both testosterone and the more potent androgen dihydrotestosterone (DHT) occurring in response to sprinting. This served as a backdrop to study 4 (Chapter 6) which examined the impact of nutritional ketosis on sprint performance and showed that ketone ester consumption impaired repeat sprint cycling performance (average sprint power, KET v CHO:  $507.3 \pm 73.3\text{W}$  v  $518.1 \pm 72.9\text{W}$ ;  $P < 0.01$ ) alongside a dampening of the hormonal response that correlated with the performance deficit ( $r = 0.538$ ;  $P = 0.047$ ). Finally study 5 (Chapter 7) explored the translation of these findings into field interventions in sport through a case study series demonstrating that dietary ketone ester consumption is achievable during sports training and competition, leading to sustained nutritional ketosis, but that this comes with a significant side effect profile, poor adherence by athletes (31% withdrew from trialling due to perceived side effects of the drink and 26% withdrew from trialling due to a perceived lack of efficacy) and equivocal effects on performance.

This work provides novel insight into the metabolic effects of nutritional ketosis in highly trained athletes whilst also highlighting the importance of taking a wider psycho-physiological perspective when translating experimental findings into application for real world sports performance. When considering the potential utility of ketone ester consumption during exercise there may be a narrow window between efficacy and intolerance and much to be determined regarding the conditions under which nutritional ketosis can impact sports performance.

## Chapter 1: General Introduction

### *Physiological Limitations of Exercise Performance*

In addressing the long-standing question of where the physiological limits of exercise performance might lie, Michael Joyner joined the late Bengt Saltin in 2008 by stating that “the main regulatory and adaptive responses to acute and chronic exercise defy simple reductionist explanations” (Joyner & Saltin, 2008). Saltin had done more than most, in the decades prior, to elaborate a view that whole-body aerobic capacity is a primary limiter of endurance exercise and further that this capacity is limited by the circulation of oxygen to the periphery during whole-body exercise (Boushel, 2017). Endurance exercise performance is now understood at the physiological level to be more fully, if not completely, explained by the integrated functioning of the aerobic capacity, the lactate threshold and movement efficiency (Joyner & Coyle, 2008).

Beyond this energetic approach, much has been done to elaborate a wide array of aspects of integrative physiology, such as temperature regulation, both whole-body and local tissue O<sub>2</sub> kinetics and fuel availability, that may be brought to bear on exercise performance in a context dependent manner (Tucker & Noakes, 2009). The recognition that physiological systems mount an integrative response to exercise has also led to increasing attention being paid to the anticipatory regulation of performance through afferent feedback (Atkinson, Peacock, St Clair Gibson, & Tucker, 2007) and the role of effort perception in co-ordinating the physiological response to exercise (Tucker, 2009).

The multiplicity of factors, both known and unknown, that influence the physiological limits of exercise performance has led to many and varied attempts to influence exercise performance by physiological means. Efforts to nutritionally impact the biochemistry of energetic substrate selection and utility have been a mainstay of such approaches and continue to offer new promise (Beneke & Boning, 2008).

### *The Selection of Energetic Substrate*

The regulation of mitochondrial substrate has been the subject of intense speculation ever since the concept of a glucose fatty-acid cycle was first introduced by Sir Phillip Randle and colleagues in 1963 (P.J. Randle, Garland, Hales, & Newsholme, 1963). In its original form the glucose fatty-acid cycle proposed that the beta oxidation of circulating free fatty acids exerts an inhibitory influence on glucose oxidation via the combined effects of inhibition of phosphofructokinase (PFK) by accumulating citrate and inhibition of pyruvate dehydrogenase (PDH) by accumulating AcetylCoA. This model has since been reaffirmed in an updated and revised form to include various aspects of enzymatic and cell signalling control, which were not known at that time (Philip J. Randle, 1998). Despite this, the role of the glucose fatty-acid cycle as a mainstay in the regulation of substrate switching has been challenged, particularly in relation to the insulin resistance of diabetes (Kelley & Mandarino, 2000). However, whilst the complex dynamics of substrate control require further clarification, it is well established that fat is the preferred fuel source for cellular energetics at rest in healthy humans, indicating the default position of mitochondrial substrate selection (Rolfe & Brown, 1997). The higher free energy yield

of fat per carbon unit and the energy density of fat as a storage substrate appear the most likely evolutionary explanations for this preference (Rolls, 2000).

During exercise, there is a clear switch in substrate preference towards reliance upon carbohydrate as energetic demand increases and oxygen abundance diminishes, reflecting the need for an improved P/O ratio (Fontaine & Leverve, 2001). In the fasted condition exercising muscle demonstrates a predictable shift in substrate selection with increasing exercise intensity (JA Romijn, Gastaldelli, Horowitz, Endert, & Wolfe, 1993; Van Loon, Greenhaff, Constantin-Teodosiu, Saris, & Wagenmakers, 2001) such that fat is preferred as a fuel source at low intensity, its oxidation rate peaking at around 65%  $VO_{2max}$ , whilst the role of carbohydrate increases continuously as exercise intensity goes up and the rate of demand for high energy phosphates increases. There is now growing evidence to support a predominant role for both local contractile mechanics and local tissue energetics in the signalling cascade that promotes an increase in exercising mitochondrial carbohydrate oxidation (Rose & Richter, 2005). Further to this, it would appear that this increased glycolytic flux exerts an inhibitory effect on beta oxidation through its influence on carnitine shuttling across the mitochondrial membrane, suggesting that the exercising muscle cell is able to reverse the preference for lipolytic substrate seen in resting mitochondria (Clarkson et al., 2002).

It is perhaps surprising then, given the complex regulation of mitochondrial substrate selection, that the oxidation of substrate by active muscle can be significantly altered by acute variation in dietary substrate delivery, exerting modulatory effects on

muscle energetics during exercise (Hargreaves, Hawley, & Jeukendrup, 2004). It has long been known that carbohydrate feeding before (Bergström, Hermansen, Hultman, & Saltin, 1967) or during (Coff, 1983) prolonged exhaustive exercise can promote glycogen sparing and extend muscular work and time to fatigue. Dietary fat feeding has also been shown to exert glycogen sparing effects (Vukovich et al., 1993) with a concomitant impact on endurance performance in exercising rats (Hickson, Rennie, Conlee, Winder, & Holloszy, 1977) although this finding has not been consistently replicated in humans (Hargreaves et al., 2004). Endurance exercise training itself confers a degree of glycogen sparing by increasing reliance upon endogenous fat as an energetic substrate at comparative exercise intensities (Hermansen, Hultman, & Saltin, 1967). This substrate switching appears to be highly adaptable to changes in demand with just 8 weeks of moderate exercise performed 3-4 times/week capable of provoking a profound shift in exercising leg RQ (from 0.91 to 0.81) indicating a near 40% swing from carbohydrate to fat oxidation (Kiens, Essen-Gustavsson, Christensen, & Saltin, 1993).

Despite the ability of dietary carbohydrate to replace stored glycogen as a source of fuel for muscular work, it does not appear to impact upon exercising RER (Coff, 1983), suggesting that carbohydrate feeding does not significantly alter mitochondrial substrate selection during exercise. By contrast, acute high fat feeding can increase total fat oxidation by a third during moderate intensity exercise, even when carbohydrate is the predominant fuel source (Pitsiladis, Smith, & Maughan, 1999). In this study fat feeding resulted in an 8% improvement in time to exhaustion during a ~2hr performance trial, despite a relatively small increase in the contribution of fat



to total oxidation (less than 10% swing in substrate selection) with a modest drop in RER ( $<0.02$  units) which only significantly differed from a carbohydrate feeding trial in the early part of this prolonged bout of exercise. However, with lower intensities of exercise and more prolonged changes in dietary substrate delivery, following a week long high fat diet, substrate selection has been shown to alter further still, as evidenced by changes in exercising RER in excess of 0.05 units (Horner et al., 2002). This pronounced alteration in the carbon source for oxidation towards a greater reliance on fat exposed a worsening of gross efficiency during exercise in that study, which was attributed not only to the relatively low P/O stoichiometry of fat oxidation, but to a larger extent was related to a significant increase in uncoupled respiration following high fat feeding.

The observation that fat oxidation carries an additional oxygen cost, which is not coupled to phosphate delivery, has attracted much recent interest (Fontaine & Leverve, 2001). The degree of uncoupled respiration at rest has been estimated to be as much as 20% of whole body mitochondrial oxidation (Rolfe & Brown, 1997), and varies in proportion to the selection of fat as an oxidative substrate. Far less is currently understood about the dynamics of mitochondrial uncoupling during exercise, although it is known that exercise training promotes a reduction in uncoupling protein 3 (UCP3) levels in muscle mitochondria (Fernström, Tonkonogi, & Sahlin, 2004), with a concomitant improvement in energetic efficiency during exercise (Schrauwen, Troost, Xia, Ravussin, & Saris, 1999).

The influence of substrate selection on the energetics of rest-exercise transients also remains largely unexplored. Exercise training has been shown to hasten the time course of engagement of mitochondrial respiration, as evidenced by more rapid on-kinetics for pulmonary  $\text{VO}_2$  (S J Bailey, Vanhatalo, Wilkerson, Dimenna, & Jones, 2009). However, the influence of substrate selection on  $\text{VO}_2$  kinetics requires some clarification. It is known, for example, that pharmacological activation of the PDH complex by dichloroacetate (DCA) can increase mitochondrial glycolytic flux at the onset of exercise even when oxygen supply is low, suggesting that a lag exists in the engagement of carbohydrate oxidation (Timmons et al., 1998). Despite this apparent glycolytic inertia there is only limited evidence of a concurrent reliance upon fat oxidation in the early exercise transition (Molé & Hoffmann, 1999). Furthermore, it has also been shown that PDH activation by DCA causes an apparent slowing of phase two  $\text{VO}_2$  kinetics that appears contradictory to the effects of exercise training (Marwood et al., 2010). Crucially, however, this slowing does not appear to be accompanied by an increase in anaerobic work as no net increase in lactate efflux is present following DCA infusion.

#### *Monocarboxylate Theory of Substrate Supply at High Rates of Aerobic Demand*

The standing of lactic acid as a negative by-product of muscular work in the absence of oxygen was a mainstay of work physiology until the latter part of the 20<sup>th</sup> century. However, it is now well established that “lactate is formed and utilised continuously under fully aerobic conditions” (Brooks, 2002). Much work has now been done to establish lactate as a dynamically regulated substrate that responds to energetic need through inter-cell and intra-cellular lactate shuttles to ensure the rapid

availability of oxidative substrate to working muscle, particularly during periods of high demand (Brooks, 2009; Emhoff et al., 2013). Recent estimates suggest that lactate may in fact be the dominant mitochondrial substrate during high intensity exercise and further that lactate may also serve as an important substrate for the brain (Rasmussen, Wyss, & Lundby, 2011; van Hall et al., 2009).

The dynamic interplay between lactate and pyruvate, regulated by lactate dehydrogenase, plays an important role in regulating cellular redox state (G. A. Brooks, Dubouchaud, Brown, Sicurello, & Butz, 1999). The removal of lactate from sites of accumulation such as active type II muscle fibres serves both to stabilise the redox environment for those cells and also to signal this changing redox state by altering redox balance in the cells of lactate uptake such as type I muscle fibres (G A Brooks, 2007).

In addition to serving a role in redox balance for active tissues the presence of high rates of lactate flux serves to ensure that a rapidly combustible energy source is available during high levels of aerobic energy provision. The presence of active transporters for cellular lactate shuttling ensures the management of concentration and redox gradients that optimise the efficiency of working muscle by channelling lactate delivery to sites of need (G A Brooks, 2007). In conditions favouring both a high rate of ATP turnover and optimally efficient P/O ratios monocarboxylates may offer a supply line uniquely adapted to this need (Chatham, 2002). From this perspective the accumulation of lactate with increasing intensity of exercise can be seen as a desirable rather than undesirable response to the demands of the task.

That the adaptation to such work with an exercise training programme relies upon mechanisms that facilitate lactate transport is in support of this view (Bickham, Bentley, Le Rossignol, & Cameron-Smith, 2006).

Given the complex dynamics of substrate selection during exercise, the introduction of a fourth major substrate in the form of nutritional ketosis is an intriguing prospect. Early evidence indicates that ketone bodies may be hierarchically selected over other substrates when available for oxidation by cells with a high energy demand such as cardiac muscle, with this alteration in substrate selection conferring energetic benefits in the form of an increased hydraulic efficiency for the working rat heart (Kashiwaya, King, & Veech, 1997). Whether similar benefits can be conferred to working skeletal muscle is worthy of further investigation.

#### *Historical view of Ketone Body Metabolism*

In eucaloric states supported by abundant carbohydrate supply this substrate is the preferred fuel source both for cerebral energetics and for high intensity muscular work. In states of severe or prolonged energetic restriction however, declining carbohydrate reserves provoke an increase in hepatic production of ketone bodies from lipid precursors in order to provide an alternate supply of substrate for mitochondrial oxidation that does not depend on the beta-oxidation of fats (Cahill et al., 1966). Further still in uncontrolled diabetes, episodes of profound hypoglycaemia are accompanied by a compensatory ketoacidosis that can raise levels of circulating ketone bodies to potentially lethal levels due to the associated acid load (Kitabchi, Umpierrez, Murphy, & Kreisberg, 2006). As a result the physiology of ketone bodies

has long been associated with the morbidity of extreme energetic restriction and pathological states.

#### *Emerging Role of Ketone Bodies in a Eucaloric State*

Despite their long held position as a fuel of last resort under energetic compromise, it has long been known that ketone body production is readily inducible through dietary carbohydrate restriction (Koeslag, 1982) in the absence of overall energy deficit. Whilst uncommon in the post-agricultural modern day diet, it appears likely that for much of the evolutionary history of modern man, periods of enforced energetic restriction due to a lack of food availability were interspersed with plentiful food supply, but with seasonal variation in food sources that likely varied in their carbohydrate content such that prolonged periods could be spent achieving sustenance from high fat and protein sources, such as meat and fish (Cordain, 2002). This evolutionary backdrop indicates that ketotic states may have been far more common for historical man than they are today.

Although severe carbohydrate restriction is required to induce ketosis at rest, presumably serving to maintain adequate substrate supply to the brain, it would appear that the heightened energetic demands of exercise are capable of augmenting this response (Koeslag, Noakes, & Sloan, 1980). Given the benefits of a readily available monocarboxylate pool to highly aerobically active muscle cells described previously this may come as no surprise. An upregulation of ketogenesis in the absence of carbohydrate abundance may well serve to allow the maintenance of



a higher level of aerobic ATP turnover for working muscle and other energetically active tissues but this remains unproven.

### *Ketone Body Therapeutics*

It has been reported that ketone bodies may be a more efficient energy source than glucose (L. Veech, Britton Chance, Yoshihiro, 2001), provoking significant interest in their potential therapeutic use. A diet inducing mild ketosis, via carbohydrate restriction (ketogenic diet), has been reported to improve movement disorder, within days, in patients with Parkinson's disease (Jabre & Bejjani, 2006) and to reduce the frequency of seizures in patients with epilepsy (Kossoff, 2004). A ketogenic diet has also shown therapeutic benefit in diabetes, both in rodents (Poplawski et al., 2011) and humans (Yancy et al., 2005). However, a major issue with ketogenic diets is that strict carbohydrate restriction (<20 g/day) must be enforced to raise circulating ketone body concentration. The high fat and low carbohydrate content make the diet unpleasant, with the result that ketogenic diets are poorly tolerated and have been linked to side effects such as increased plasma atherogenic lipoproteins, constipation, kidney stones and gallstones (Kwiterovich, Jr et al., 2003; McNally, Pyzik, Rubenstein, Hamdy, & Kossoff, 2009).

### *Butanediol and Medium Chain Triglycerides as Ketogenic Agents*

Given the difficulties associated with ketogenic diets a number of attempts have been made to supplement the diet with metabolic precursors of ketone bodies. Military interest in butanediol stretches back as far as the second world war with a great deal of research in the decades that followed centred on exploring the utility

and practicalities of dietary supplementation with this ketogenic precursor (Schussel, 1953). Several animal studies have shown the efficacy of butanediol as a ketogenic agent and indicated potential for therapeutic gain (Dymsza, 1975). However, difficulty in achieving sufficient dose for sustainable ketosis coupled with potential side effects with high level dosing, particularly with regard to alcohol induced depression of CNS activity (Frye et al., 1981), led to dwindling interest in this approach.

The ketogenic potential of medium chain triglycerides has been examined with reports suggesting that tolerable levels of dietary MCTs are capable of inducing a mild ketosis approaching 1mM BHB. The benefits of this approach are currently the subject of trials in a variety of therapeutic domains.

### *Ketone Metabolism*

When ketone bodies are elevated by continuous infusion in the post absorptive state, the ensuing moderate ketosis (beta-hydroxybutyrate: 1.7 mmol/l) exhibits significant and rapid (within 30-minutes) effects on metabolism including a  $14 \pm 2\%$  reduction in glucose appearance and  $37 \pm 4\%$  reduction in circulating glycerol with no concomitant changes in insulin or glucagon (Mikkelsen, Seifert, Secher, Grondal, & Van Hall, 2015). Further, this altered metabolic state resulted in the net uptake and oxidation of ketone bodies by both brain and muscle in resting healthy men. Early indications are that exercise can further upregulate the uptake and utilisation of ketone bodies in muscle under similarly ketotic conditions induced by consumption of a ketone ester (Cox et al., 2016).

### *Development of a Ketone Ester*

Recent attempts to induce nutritional ketosis have centred on the use of esterification as a delivery vehicle (Desrochers et al., 1995). A ketone ester formed of acetoacetate and the ketogenic precursor butanediol (R,S-butanediol diacetoacetate) has been shown to incur rapid hydrolysis by tissue and circulating esterases liberating R,S-butanediol and acetoacetate into circulation. R,S-butanediol undergoes hepatic clearance via oxidation to R,S-beta-hydroxybutyrate and acetoacetate is reduced in the liver to form further R-beta-hydroxybutyrate. R,S-butanediol diacetoacetate has been successfully used in the dog to supplant up to 50% of daily caloric requirement resulting in a sustained ketosis (Puchowicz et al., 2000). An R-1,3,-butanediol D-beta-hydroxybutyrate ester fed at 30% of daily caloric intake for 14 days to normal Wistar rats provoked significant alterations in cerebral metabolism and resulted in reductions in voluntary food intake (Kashiwaya et al., 2010). The same ester has also been shown to reduce amyloid plaque and hyperphosphorylated tau deposition and to ameliorate cognitive deficits in a mouse model of Alzheimer's disease (Kashiwaya et al., 2013). In humans R-1,3,-butanediol D-beta-hydroxybutyrate ester fed at varying doses up to 30% of daily caloric intake for 5 days induced dose dependent increases in circulating ketone bodies with peak dose (beta-hydroxybutyrate: 3.3 mmol/l) occurring 1.5-2.5 hours after an acute bolus (Clarke et al., 2012). However, whilst the ester was generally tolerated well, significant gastrointestinal side effects were reported at the highest dose delivered.

It is now possible to rapidly raise circulating ketone body concentration, in a dose dependent fashion by providing ketone esters in a drink that can be consumed as part of a normal diet. This may offer the same benefits as the ketogenic diet, without the need for severe carbohydrate restriction. Since the ketone ester drink provides an alternative source of energy with potential benefits to metabolism, it is of great interest to investigate its role as an energy drink during exercise.

#### *Ketone Ester Consumption as a Novel Nutritional Intervention for Sports Performance*

Acute dietary supplementation with a ketone ester drink is capable of inducing a sustained ketosis that has wide ranging effects on substrate selection, exercising energetics and endurance performance (Cox et al., 2016). Nutritional ketosis has further been shown to improve performance in elite rowers performing a 30-minute rowing ergometer time-trial (Cox, 2012) and in well-trained rugby players during a 96-minute rugby match-play simulation protocol (unpublished data from our group). The wider conditions for ergogenic benefit of nutritional ketosis in sport are not yet known. The modes, durations and intensities of exercise where benefit may be seen and the factors influencing efficacy, such as dosing regimens and favourable phenotypical characteristics remain to be determined.

#### *Behavioural Biology of Pacing for Sports Performance*

Optimal competition performance in many sports requires the behavioural regulation of instantaneous work output to pace the expenditure of resources such that the overall requirements of the task are met with maximal efficiency (Renfree, Martin, Richards, St, & Gibson, 2012). Whether the task requirements are continuous

or intermittent, for all but the shortest and most explosive sports there is performance benefit to be gained from some level of regulation of resource expenditure over attempts to work maximally from task onset to task completion (Abbiss & Laursen, 2008). When pacing is ineffective a catastrophic loss of work output can result prior to task completion, particularly when the disturbance to homeostasis is profound enough to threaten harm if exercise were to be continued at the same intensity (Tattersson, Hahn, Martini, & Febbraio, 2000).

Whilst homeostatic perturbations are highly task specific there is a high degree of confluence in their regulatory control once the task demands are set. It appears that effective resource allocation requires both an accurate estimate of the demands of the task and an effective gauge of ongoing resource expenditure (Tucker, 2009). However, given that exercise is almost always curtailed before actual harm ensues a further key ingredient in pacing strategy is motivation to task. Exemplifying this a number of studies have demonstrated that subversive manipulation of the perceived balance of task demands and resource availability can alter actual work output to impact overall performance either when demands are underestimated (false feedback; Ansley et al. 2004) or resources are overestimated (carbohydrate mouth rinse; Carter et al. 2004).

For sustained endurance tasks a number of studies in a variety of contexts have demonstrated that fuel status and thermal load are key determinants of resource allocation with pacing strategy being determined by a desire to avoid the upper limits of body temperature or lower limits of muscle glycogen storage (Tucker & Noakes, 2009). To this end a great number of attempts have been made to confer ergogenic



gain to exercise performance by dampening heat stress or enhancing fuel supply through the provision of exogenous substrate (Burke, Hawley, Wong, & Jeukendrup, 2011).

Pacing behaviours have also been identified during more intense sustained exercise tasks as short as 30-seconds in duration and are thought to be associated with the need to maintain within tolerable limits homeostatic disturbances to regulatory aspects of physiology such as acid-base status (Cairns, 2006). Amongst the array of training induced adaptations to high intensity exercise, elevations in base excess via the accumulation of intra- and extra-cellular buffering reserves (Gibala et al., 2006) and improvements in mitochondrial lactate handling via the up regulation of monocarboxylate transporters (MCTs; Bickham et al. 2006) are key factors in supporting the maintenance of acid-base status during prolonged high intensity work in order to sustain an elevated pacing strategy for optimal performance.

#### *The Ecological Dynamics of Novel Nutritional Interventions in Sport*

The ecological validity of utilising laboratory trial data to evidence the ergogenic potential of performance interventions for translation onto the sports field has come under increasing scrutiny given the growing mass of trial data that now influences sporting practice (Atkinson & Nevill, 2001). Whilst laboratory trials have obvious utility in providing a highly controlled environment for the assessment of putative ergogenic interventions, there are a number of aspects of the trial environment and context that are worthy of consideration when translating the value of a given finding into a given sporting scenario. Viewed from the perspective of dynamical systems

theory (Phillips, Davids, Renshaw, & Portus, 2010) each performance ecology is constrained by the shifting dynamics of the performer, the task at hand and the performance environment. The nature of, and interactions between, these constraints being a key consideration in determining the relationship between what emerges in the laboratory and what is possible in the field.

The training status of trial participants is an often overlooked constraint on the ergogenic potential of nutritional interventions for sports performance. By way of example the utility of dietary nitrate, most commonly through the consumption of beetroot juice was well established as having ergogenic potential in untrained or moderately trained populations (Stephen J Bailey et al., 2009; Lansley, Winyard, Bailey, et al., 2011; Lansley, Winyard, Fulford, et al., 2011), and in common usage amongst competitive athletes (Lundberg, Larsen, & Weitzberg, 2011) for some time before evidence started to emerge suggesting that a highly trained athletic population may not benefit from such intervention (Bescós et al., 2012; Christensen, Nyberg, & Bangsbo, 2013; Peacock et al., 2012), most likely as a result of having already optimised through regular training the physiological advantages on offer. Indeed, similar questions have recently been asked of nutritional ketosis given that levels of interest perhaps outweigh the available evidence (Pinckaers, Churchward-Venne, Bailey, & van Loon, 2016).

Likewise, it is common in laboratory trials to adopt exercise protocols that only loosely reflect the actual demands of sport in an effort to provide a controllable and repeatable stimulus for assessment of ergogenic potential (Atkinson & Nevill, 2001).

Whilst this places some limitation on the translation of laboratory findings to a given sporting context, the factor most likely to impact the ecological validity of laboratory assessments of volitional work capacity is the motivational climate surrounding the performance itself (Buch, Nerstad, Aandstad, & Säfvenbom, 2016). Elite athletes are known to exhibit both performance and mastery orientation to achievement goals (Pensgaard & Roberts, 2000) and are likely to adapt differently to a motivational climate that emphasises one or other rather than both of these orientations. If laboratory work is to translate to a high performance sporting context it would appear desirable that the motivational climate should present a level of challenge that addresses these needs (Corbett, Barwood, Ouzounoglou, Thelwell, & Dicks, 2012a) but this is seldom the case for the laboratory assessment of novel nutritional interventions.

In seeking to better understand the complex dynamics of sporting performance and the psycho-physiology that underpins them, the endocrine system may serve as an elegant guide. Hormonal signalling through the hypothalamic-pituitary-adrenal (HPA) axis has been shown to exert a modulatory influence over both functional and adaptive responses to exercise stimuli (Crewther, Cook, Cardinale, Weatherby, & Lowe, 2011). Whilst the majority of work in this area has focussed on the influence of HPA axis activity on strength and power adaptation to exercise training (Hakkinen & Pakarinen, 1995; Kraemer & Ratamess, 2005), a growing body of evidence is converging on a critical role for the interplay of androgen and glucocorticoid signalling in supporting an optimal motivational climate for sports performance (Cook & Crewther, 2012a, 2012b). Early indications are that these effects may be of

particular import for the female athlete (Cook, Crewther, & Smith, 2012) but more generally much remains to be determined in elucidating the role of androgen and glucocorticoid signalling in modulating sports performance.

### *Thesis Aims*

There is now significant interest in the potential ergogenic benefits of nutritional ketosis born of some promising but limited early laboratory findings demonstrating a small positive effect on sustained endurance performance in both rowing (Cox, 2012) and cycling (Cox et al., 2016). This thesis aims to inform the utility of nutritional ketosis for sports performance by exploring the translation of these early experimental findings regarding ketone ester consumption into both laboratory and field work with increasing ecological validity to the sports person. Experimental studies will examine the practical factors impacting the pharmacokinetic response to ketone ester dosing, such as meal timing and warm up exercise, as well as the performance impact of lowering the dose of ketone ester consumed in an attempt to improve gastro-intestinal tolerance of the drink (Clarke et al., 2012). The impact of nutritional ketosis on repeat sprint performance will be examined to further the early work that has focussed on sustained endurance exercise. As a backdrop to this, and in order to explore the behavioural biology surrounding this novel nutritional intervention, the hormonal response to repeat sprint exercise will first be characterised in a cohort of well trained athletes. Finally, efforts to case study ketone ester consumption during real-world sports training

and competition will be described to highlight some of the practical challenges and potential issues to consider in exploring the utility of this novel nutritional state.

## Chapter 2: General Methods

### *Drink formulations and safety*

Experimental beverages containing D-β-hydroxybutyrate-R-1,3-butanediol, a novel ketone monoester (dG: TΔS, Oxford, UK), were developed in collaboration with a nutritional product development specialist, the International Food Network (IFN, Reading, UK). Each beverage formulation contained varying concentrations of ketone ester (6-10mg/min/kgBW) and calorie matched carbohydrate (dextrose and fructose in a 2:1 ratio) as detailed in each study chapter as well as additional colourings (Lutein Yellow; Naturex, Swadlincote, UK), and flavourings (Orange flavouring (650961) & Pineapple flavouring (651757); Symrise, Marlow, UK). Non-ketone ester containing control beverages were prepared following similar procedures with the addition of bitter agents to match the flavour of the ketone ester beverage and with the provision of additional carbohydrate to achieve isocaloric matching. All beverages proceeded through a product development lifecycle involving prototype development, safety and stability assessment and batch production. Following production, every batch was subjected to a strict battery of safety and quality control tests, including detailed microbial analysis and physical and sensory screening, prior to distribution and consumption. The preparation facilities at IFN were examined to ensure compliance with the standards required for Informed Sport certification as a contaminant free production facility and every batch of end-product was screened by a WADA accredited laboratory (HFL, Cambridge, UK) and shown to be free of contamination with substances on the WADA prohibited list.



In order to establish the stability and shelf life of ketone ester beverages selected batches of end-product were subjected to a series of assessments. At regular intervals over a 32-week period beverages stored in both refrigerated (8°C) and ambient (20°C) conditions underwent microbial screening and nutritional, physical and sensory analysis as well as being screened by gas chromatography mass spectrometry (GC-MS) for stability of the ketone ester. All beverages tested demonstrated microbial stability and were safe for consumption following storage in both conditions across the study period and there was no evidence of significant ketone ester degradation. Beverages stored in the ambient condition were observed to change in colour and odour over time and therefore all beverages used in this research programme were stored in refrigerated conditions (~4-8°C) at all times, except on the day of consumption.

The ketone ester was developed by Oxford University (UK) in collaboration with the National Institutes for Health (NIH, Bethesda, USA) and has been FDA (Silver Spring, USA) certified as 'Generally Recognised as Safe' (GRAS). This GRAS certification permits human consumption of the ketone ester as a foodstuff within the context of continuing research into its efficacy but does not permit general consumption or commercial sale. Given the novel nature of the ketone ester UK Sport, as the research sponsor, obtained clarification via the UK Anti-Doping Agency (UKAD) from the World Anti-Doping Agency (WADA) regarding its status in relation to the WADA code. Following the submission of a detailed dossier, including the GRAS documentation, WADA's responses stated that there was no reason on the available evidence to consider the ketone ester in contravention of the WADA code.

### *Cycle Ergometry*

All cycle ergometry, except for that used in the repeat sprint trial following pre-fatigue, was conducted on an electro-magnetically braked stationary cycle ergometer (SRM Ergometer equipped with a Science PowerMeter, Schoberer Rad Messtechnik, Jülich, Germany). The ergometer was calibrated before and after each trial in a modified version of published guidelines (Wooles, Robinson, & Keen, 2005). Manufacturer reported measurement error for the Science PowerMeter is  $\pm 0.5\%$ . However, a more rigorous calibration standard of less than  $\pm 0.1\%$  can be achieved with further control of the calibration procedure. The method of calibration employed here adopted elements of the published method, such as making multiple measurements at multiple crank angles, but did not employ a precision machined chain ring and measured output from the PowerMeter in situ mounted on the ergometer rather than unmounted and fixed in a bench-top clamp. As a result, it is likely that the achieved accuracy of the method chosen lay somewhere between the two reported values above.

The microprocessor controlled braking system on the SRM ergometer allowed for two modes of operation to be employed. In open mode the ergometer mimicked a regular bicycle with a fixed magnetic resistance complimented by the presence of a standard bicycle cassette that allowed the rider to select gearing and cadence in order to self-regulate power output. In pre-determined mode the gear was fixed and electro-magnetic resistance was constantly adjusted to compensate for variation in cycling cadence in order to maintain a computer regulated pre-set power output as dictated by the software controlling the microprocessor. The SRM software collected

data on every pedal stroke from the ergometer allowing for both real time graphical feedback and post-hoc segmentation and analysis of test data files.

For the repeat sprint study following pre-fatigue the assessment of participants in groups of up to eight riders at a time necessitated the use of an alternate bicycle ergometer. These trials were conducted on air-braked stationary cycle ergometers (WattBike Pro, Nottingham, UK). WattBikes were factory calibrated with manufacturer reported accuracy better than  $\pm 2.0\%$  in the range 100-200W, better than  $\pm 1.5\%$  in the range 200-500W and better than  $\pm 1.0\%$  at power outputs above 500W. Test-retest reliability of an individual 30-second maximal sprint on the WattBike has been examined in trained cyclists with a reported CV of 2.4% for mean power (Driller, Argus, & Shing, 2013). Individual participants were allocated a set ergometer to use repeatedly for all laboratory visits in order to minimise test-retest measurement error.

For both ergometers (SRM Ergometer & WattBike Pro) each participant's riding position was customised to individual rider preference by adjusting the horizontal and vertical alignment of both the saddle and the handlebars. The preferred setup was recorded on the first visited and replicated for all subsequent visits to the laboratory. Participants were afforded the opportunity to use their own pedals and cycling shoes and if required their own saddle. All rider customisations were recorded and repeated on each laboratory visit.

### *Respired Gas exchange*

At designated time intervals participants were required to breathe through a rubber mouthpiece attached to a two-way non-rebreathing valve (Hans Rudolph, Kansas City, USA) and expired gases were collected using high bore low resistance tubing connected to a Douglas bag via a stopcock that allowed timed collections of expired air. Gas collections were analysed for volume of air expired using a dry gas meter (Harvard Apparatus, Kent, UK), temperature of expired gases via a digital thermometer (model C, Edale Instruments, Cambridge, UK) and fractional concentrations of O<sub>2</sub> and CO<sub>2</sub> using paramagnetic and infrared methods, respectively (Servoflex MiniMP, Servomex, Crowborough, UK). Barometric pressure was recorded at the point in time when gas analysis took place. Analysers were calibrated prior to each test with gases of known composition within the physiological range, as certified by prior gravimetric analysis (British Oxygen Company, UK). Douglas bags were checked for leakage and evacuated under negative pressure prior to each usage. Measured values were used to determine ventilatory rate ( $V_E$ ), the rate of oxygen consumption ( $VO_2$ ) and the rate of carbon dioxide production ( $VCO_2$ ) and corrected to standard conditions (standard temperature and pressure, dry; STPD) for comparison across trials.

### *Blood collection*

Capillary blood samples were collected from an earlobe as designated in the trial schedule. Prior to each dermal puncture a site was selected and prepared by thorough abrasion with an alcohol swab (Molnlycke Sterets, Gothenburg, Sweden) to remove surface debris from the puncture site. Dermal puncture was achieved by

rapid insertion and withdrawal of a sterile lancet (Accu-Chek Safe-T-Pro, Roche, Basel, Switzerland) with the earlobe held under tension. Free flowing blood was collected from the puncture site either by capillary action as described below or by passive draw into a 0.5ml EDTA sample bucket (Microvette 500, Sarstedt, Nümbrecht, Germany). EDTA samples were refrigerated immediately and centrifuged (Heraeus Biofuge Pico, Thermo-Scientific, Waltham, USA) for 10 minutes as soon as possible after collection. The plasma portion of each sample was extracted using a positive displacement pipette (Biohit Proline Plus Mechanical Pipette, Sartorius, Helsinki, Finland) and redistributed into polypropylene sample tubes (1.5ml Microcentrifuge Safe-Lock Tubes, Fischer Scientific, Loughborough, UK). Following extraction samples were stored at -80°C until analysis.

Venous blood samples were collected either by discrete venepuncture or by insertion of a venous cannula depending on the number of repeated samples required on a given visit to the laboratory. For either procedure a tourniquet was placed on the upper arm and adjusted to a level of tightness that encouraged protrusion of the distal superficial veins without causing loss of sensation or excessive discomfort. A suitable antecubital vein was identified and the puncture site was prepared by thorough abrasion with an alcohol swab (Molnlycke Sterets, Gothenburg, Sweden) to remove surface debris.

For the venepuncture procedure a butterfly needle with a syringe attached (Vacutainer Safety-Lok Blood Collection Set, BD, New Jersey, USA) was advanced through the skin and into the designated vein. Flush back of blood into the butterfly

tubing was used to guide the insertion of the needle. Once the needle was in situ the tourniquet was released and with the butterfly held in place against the skin a 5ml blood sample was drawn into the syringe. The needle was then immediately withdrawn and manual pressure applied to the puncture site for at least two minutes or until the puncture had sealed.

For the cannulation procedure a venous cannula needle (Venflon Pro, BD, New Jersey, USA) was advanced through the skin and into the designated vein. Flush back of blood into the cannula sheath was used to guide the insertion of the needle. Once the needle was in situ the cannula was advanced into the vein whilst holding the needle in place. With the vein manually occluded proximal to the puncture site the needle was then withdrawn and a sample line with a 3-way tap (Connecta 3-Way Stopcock, BD, New Jersey, USA) was attached to the cannula before the occlusion was released. The cannula was secured to the skin with an adhesive (Veca-C, BD, New Jersey, USA) and the tourniquet released. A 5ml blood sample was drawn into a syringe attached to the 3-way tap and the line was then flushed with 2ml of saline (Sodium Chloride BP, B.Braun, Sheffield, UK) from a second syringe. All subsequent sample collections from the cannula involved withdrawal and discarding of the ~1ml of fluid contained in the tubing immediately prior to collection of a 5ml sample. Following every sample collection 2ml of saline was inserted into the line to preserve the patency of the cannula.

Venous samples were immediately transferred into either EDTA (EDTA KE/5ml, Sarstedt, Nümbrecht, Germany) or serum (Serum Gel/5ml, Sarstedt, Nümbrecht,



Germany) collection tubes. EDTA samples were refrigerated immediately and centrifuged (Heraeus Biofuge Primo R, Thermo-Scientific, Waltham, USA) for 10 minutes as soon as possible after collection. The plasma portion of each sample was extracted using a positive displacement pipette (Biohit Proline Plus Mechanical Pipette, Sartorius, Helsinki, Finland) and redistributed into polypropylene sample tubes (1.5ml Microcentrifuge Safe-Lock Tubes, Fischer Scientific, Loughborough, UK). Serum samples were left at room temperature for 15 minutes and then centrifuged (Heraeus Biofuge Primo R, Thermo-Scientific, Waltham, USA) at 1500g for 10 minutes. The serum portion of each sample was extracted using a positive displacement pipette (Biohit Proline Plus Mechanical Pipette, Sartorius, Helsinki, Finland) and redistributed into polypropylene sample tubes (1.5ml Microcentrifuge Safe-Lock Tubes, Fischer Scientific, Loughborough, UK). Following extraction all samples were stored at -80°C until analysis.

#### *Instantaneous Blood Ketone Measurement*

For some trials the measurement of blood ketone concentration was made at the point of collection using a handheld monitor employing a microfluidic strip technology (Optium Xceed and  $\beta$ -ketone strips, Abbott Laboratories Ltd, Maidenhead, UK). For capillary earlobe samples, a test strip was inserted into the monitor and the tip of the strip was presented to a blub of fresh blood formed at the base of the earlobe. A small volume of blood (10 $\mu$ l) was drawn into the strip by capillary action and the monitor reported a test result within 10 seconds. For venous samples the tip of the test strip was presented to the tip of the syringe following blood extraction but prior to any processing. This measurement method has

previously been shown to provide accurate (mean difference  $<0.1\text{mmol/l}$  compared to enzymatic reference method) and reliable (repeat measures coefficient of variation  $<5\%$ ) blood ketone concentrations for both venous and capillary samples across the physiological range up to  $5\text{mmol/l}$  (Byrne, Tieszen, Hollis, Dornan, & New, 2000).

### *Capillary Blood Gas Analysis*

Capillary samples for blood gas analysis were drawn into pre-treated heparin glass capillaries (Clinitubes, Radiometer, Brønshøj, Denmark) by capillary action from a blub of fresh blood formed at the base of the earlobe. Samples were agitated by hand for 30 seconds prior to being presented to an automated blood gas analyser (ABL 70, Radiometer, Brønshøj, Denmark). With a capillary blood sample presented to the Radiometer sample needle, measurement was initiated and the device aspirated the required sample volume for analysis ( $65\mu\text{l}$ ). Samples were presented to a series of sensors for analysis of pH,  $\text{pO}_2$  and  $\text{pCO}_2$  and measured values were used to derive estimates of blood bicarbonate concentration and base excess (Burnett & Noonan 1974). Calibration of the Radiometer ABL 70 involved an automated procedure utilising a calibration cassette pre-filled with standard solutions in the physiological range for each parameter. In addition to instrument calibration a quality control procedure was conducted prior to each test session. Four different quality control solutions across the physiological range (QualiCHECK+, Radiometer, Brønshøj, Denmark) were presented to the device and checked against factory-calibrated values to ensure that the device was operating within acceptable limits across its measurement range.

Capillary blood gas measures offer a practical surrogate for arterial blood gas analysis without the need for an invasive and painful procedure, with the earlobe the preferred site of sample collection (Zavorsky, Cao, Mayo, Gabbay, & Murias, 2007). However, the measured values demonstrate a loss of accuracy and reliability compared to the more robust method of arterial blood collection. In order to minimise measurement error only samples that recorded an SaO<sub>2</sub> of greater than 94% were included in the analysis.

### *Blood biochemistry*

On the day of analysis frozen samples were fully defrosted to room temperature (~20°C) and partitioned for analysis by multiple assays as indicated in the trial schedule. Samples were analysed for concentrations of glucose, lactate, non-esterified fatty acids (NEFA), Glycerol and  $\beta$ -hydroxybutyrate by spectrophotometry (RX Daytona, Randox, Crumlin, UK) and by commercial ELISA kits for concentrations of insulin (Merckodia, Uppsala, Sweden), total testosterone, free testosterone, dihydrotestosterone and cortisol (IBL, Hamburg, Germany). For the repeat sprint trial (Chapter 7) plasma samples were analysed for dihydrotestosterone using an alternate ELISA kit (MyBioSource, USA) due to the incompatibility of the serum analysis kit (IBL) as a result of cross reactivity between the assay enzyme conjugate and the anticoagulant in the sample.

### *Training History Questionnaire*

A self-report training history questionnaire (Appendix 1) was deployed with the aim of capturing the broad history of participation in sports and exercise training of study

participants across their lifespan. An extended section on cycling was included given the selection of cycling as a test modality for the majority of the research methods employed in the research programme.

### *Subjective Rating Scales*

Validated subjective rating scales were used for the determination of perceived effort (Appendix 2; Borg, 1970) and perceived affect (Appendix 3; Hardy & Rejeski, 1989). A drink pleasantness scale (Appendix 4; modified from Bartoshuk et al., 2004) was used to compare experimental and control drinks for tolerance and a modified gastrointestinal symptom scale (Appendix 5) was used to determine GI comfort following consumption of experimental drinks both before and during exercise (Pfeiffer, Cotterill, Grathwohl, Stellingwerff, & Jeukendrup, 2009).

### Chapter 3: The Effects of Exercise and Feeding on the Blood Ketone Body Response to Ketone Ester Consumption

#### ABSTRACT

Ketone ester consumption has been shown to alter exercise energetics and endurance performance (Cox et al., 2016) but the factors influencing ketone body availability in the real-world conditions of sport are not known. This study aimed to establish the impact of meal timing, drink composition and warm-up style exercise on the availability of circulating ketone bodies following a single dose of a ketone ester and carbohydrate drink (KET: 400mg/kg-body-mass + CHO: 480mg/kg-body-mass). Ten healthy adults (8M, 2F) consumed KET in three different conditions across two trial cohorts (feeding cohort: 90-min pre-meal (F90), 30-min pre-meal (F30), low CHO drink (KET: 400mg/kg + CHO: 120mg/kg) (F4:1); exercise cohort: rest (ER), 30-min exercise 15-min post dose (E15), 30-min exercise 45-min post dose (E45)). Ketone ester consumption resulted in a rapid rise in plasma betahydroxybutyrate (BHB) to  $3.4 \pm 0.5$  mmol/l within 60-minutes followed by an exponential decay with clearance occurring by 240-minutes post dose. Compared to F90, F4:1 resulted in a greater area under the BHB curve (AUC-BHB) by 90-mins post dose (F90 v F4:1:  $260.7 \pm 33.6$  mmol/l<sup>o</sup>min v  $311.4 \pm 49.3$  mmol/l<sup>o</sup>min;  $P < 0.05$ ) and a higher peak BHB (F90 v F4:1:  $3.5 \pm 0.5$  mmol/l v  $4.1 \pm 0.7$  mmol/l;  $P < 0.01$ ) whereas F30 result in a smaller area under the BHB curve (AUC-BHB) by 90-mins post dose (F90 v F30:  $260.7 \pm 33.6$  mmol/l<sup>o</sup>min v  $201.9 \pm 33.4$  mmol/l<sup>o</sup>min;  $P < 0.05$ ) and a trend towards lower peak BHB (F90 v F30:  $3.5 \pm 0.5$  mmol/l v  $2.8 \pm 0.4$  mmol/l;  $P = 0.07$ ). Compared to ER, neither E15 nor E45 significantly altered the AUC-BHB during the 30-min exercise bout. In

conclusion, meal timing and drink composition, but not warm-up exercise, are important considerations for dosing regimens aiming to optimise ketone body availability during exercise.



## INTRODUCTION

Many sports are characterised by high levels of energetic demand that place a functional limit on exercise tolerance and performance capacity. Amongst the factors that has long been known to influence exercise tolerance for sports performance is the provision of exogenous fuel supply to support optimal cellular energetics (Coff, 1983). Until recently, research in this area has been dominated by exploration of the dietary provision of carbohydrate and fat as exogenous substrate for exercise (Hargreaves et al., 2004) and the dietary provision of protein for post-exercise recovery (Burd, Tang, Moore, & Phillips, 2009). However, the emergence of a novel method for achieving nutritional ketosis through consumption of a ketone ester drink brings into consideration a fourth macronutrient with the potential to influence exercise metabolism (Kashiwaya et al., 2010).

Ketone ester consumption has been shown to be safe and effective at rapidly inducing a moderate ketosis under laboratory conditions (Clarke et al., 2012) and early indications suggest that nutritional ketosis may confer ergogenic benefit for sports performance under certain conditions (Cox & Clarke, 2014). However, little is known about the factors influencing ketone body availability when dosing regimens are employed under the real world conditions of sport. Factors such as timing of pre-feeding and warm-up exercise are known to influence the availability of carbohydrate during exercise (Hawley & Burke, 1997) and could have an equally important impact on ketone body availability.

One of the critical advantages of nutritional ketosis achieved via ketone ester consumption is the avoidance of the carbohydrate restriction employed in the ketogenic diet (Hashim & Vanitallie, 2014). The prospect of co-administering a ketone ester alongside carbohydrate is likely to confer benefit both to the optimisation of exercising substrate supply and to the gastric tolerance of the ketone ester drink. However, the impact of varying levels of co-dosing of ketone ester and carbohydrate on blood ketone body availability is not known.

This methodological study aims to better understand the variation in response to an acute dose of a ketone ester drink by examining the effect of meal and exercise timing and drink content on the blood ketone body response. Such information will help to optimise dosing strategies for future research and practice.

## **METHODS**

### **Participants**

Ten healthy adults participated in this study (8M, 2F; age  $32.3 \pm 5.0$  y; body mass (BM)  $77.0 \pm 14.6$  kg). The study received approval from the University of Bath Research Ethics Approval Committee for Health and each participant was provided with a written and verbal briefing regarding the nature of the testing, and provided written, informed consent prior to participation. All participants completed a health questionnaire prior to participation.

## Experimental Design

Participants were randomly assigned to one of two trial cohorts (Fig.3.1). Within each trial cohort participants were required to attend the laboratory on three separate occasions 2-7 days apart. On each occasion participants were provided with an acute dose of ketone ester under randomised conditions varying either the feeding strategy in the feeding trial cohort or the 'warm up' exercise strategy in the exercise trial cohort. Both trial cohorts contained an identical control arm allowing the characterisation of baseline responses to ketone ester consumption across all study participants.

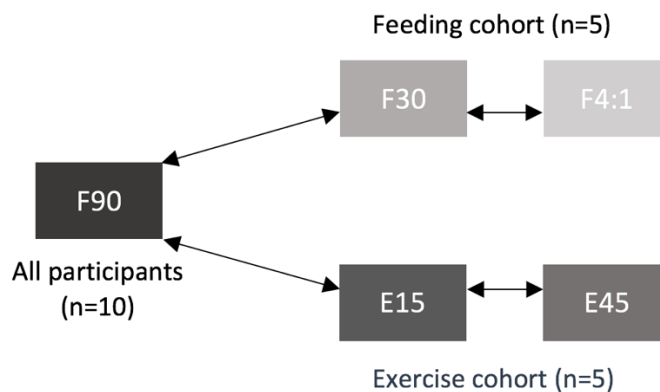


Figure 3.1. Trial schematic: participants were assigned to one of two trial cohorts. In each cohort participants completed three trials in random order with all participants completing a 90-minute pre-meal trial (F90; n=10). In the feeding cohort (n=5) participants additionally completed a 30-minute pre-meal trial (F30) and an altered dosing trial with a 4:1 ratio of ketone ester to carbohydrate (F4:1). In the exercise cohort (n=5) participants additionally completed 30-minutes of warm up exercise either 15-minutes after the ketone ester dose (E15) or 45-minutes after the ketone ester dose (E45).

## Experimental Methods

### *Trial procedures for all trials*

Participants were required to standardise their diet for the 24 hours before each trial by recording their 24-hour diet prior to the first visit and repeating this prior to subsequent visits. A first pass urine sample was collected on each trial morning to determine hydration status. Participants arrived at the laboratory after an overnight fast and were provided with a standardised breakfast at a time determined by the trial schedule.

Upon arrival at the laboratory a trained phlebotomist inserted a venous cannula into an antecubital vein without stasis. Baseline blood samples (~5ml) were drawn into EDTA collection tubes. To determine rates of appearance and disappearance of blood ketone bodies further samples were collected immediately pre ketone ester dose and at the following time points post dose: 15mins, 30mins, 45mins, 60mins, 90mins, 120mins, 150mins, 180mins, 240mins, 300mins. Blood samples were centrifuged and the plasma collected, rendering the samples acellular, and frozen at -80°C until analysis. Plasma samples were analysed for levels of betahydroxybutyrate (BHB) by spectrophotometric methods (RX Daytona, Randox, Crumlin, UK).

Following consumption of the ketone ester drink participants were required to collect all urine passed until the end of the trial into a sample container which was analysed for ketone content (Multistix 10 SG, Siemens Healthcare, Camberley, UK).

### *Feeding Trial Cohort*

In the feeding trial cohort participants completed a different trial protocol on each of three visits (Fig.3.1). Trial protocols were presented in random order.

*90-Minute Feeding Trial (F90):* Participants were provided with a standardised meal 90-minutes prior to the ketone ester dose. Ketone ester was provided as a single bolus in a drink containing a 1:1 calorie ratio of ketone ester and carbohydrate (400mg/kg-body-mass Ketone ester + 480mg/kg-body-mass carbohydrate). Participants were advised to sit at rest for the ensuing 300-minutes post dose with no exercise undertaken and no further feeding allowed. Water was provided throughout the trial period ad libitum.

*30-Minute Feeding Trial (F30):* Trial procedures were identical to trial F90 except that the standardised meal was provided 30-minutes prior to the ketone ester dose.

*Low Carbohydrate Trial (F4:1):* Trial procedures were identical to trial F90 except that the ketone ester was provided as a single bolus in a drink containing a 4:1 calorie ratio of ketone ester and carbohydrate (400mg/kg BW ketone ester + 120mg/kg BW carbohydrate).

### *Exercise Trial Cohort*

In the exercise trial cohort participants completed a different trial protocol on each of three visits (Fig.3.1). Trial protocols were presented in random order.

*90-Minute Feeding Trial (F90):* Trial procedures were identical to the F90 trial in the feeding trial cohort.

*15-Minute Exercise Trial (E15):* Trial procedures were identical to trial F90 except that 30-minutes of exercise was conducted commencing 15-minutes after the ketone ester dose. Exercise consisted of 30 minutes of sub-maximal cycle ergometry, at a self-selected intensity based on a guidance rating of perceived exertion of 11 ('Fairly Light') interspersed with four 10-second sprints at a guidance rating of perceived exertion of 17 ('Very Hard') performed at minutes 10, 15, 20 and 25 of the exercise bout. This exercise was designed to simulate the warm-up before a sports performance and the workload was repeated for the second exercise trial (E15 or E45) based on the loads selected in whichever of the trials was performed first.

*45-Minute Exercise Trial (E45):* Trial procedures were identical to trial E15 except that the 30-minute bout of exercise commenced 45-minutes after the ketone ester dose.

#### *Pharmacokinetic Modelling*

All trial data were subjected to non-compartmental analysis of plasma data after extravascular input in order to determine the pharmacokinetic parameters of plasma ketone body availability for comparison between trials (Zhang, Huo, Zhou, & Xie, 2010).



## Statistical Analysis

An analysis of variance (ANOVA) was used to identify differences between treatments for pharmacokinetic parameters, and a two-way (treatment x time) general liner model for repeated measures was used to identify differences over time in outcome variables. The Greenhouse–Geisser correction was used for epsilon <0.75, while the Huynh–Feldt correction was adopted for less severe asphericity. Where significant F values were found, the Holm–Bonferroni stepwise correction was applied to determine the location of variance. All statistical analysis was conducted using IBM SPSS Statistics (Release 20.0.0, IBM, USA) and all data are presented as mean  $\pm$  SD. Statistical significance was set at the level of  $P < 0.05$ .

## RESULTS

Time series plasma betahydroxybutyrate (BHB) responses to each trial are shown in Figure 3.1. The response profile is characterised across all participants in Trial F90 (Fig.3.1A) by a rapid rise to a peak BHB concentration of  $3.4 \pm 0.5$ mmol/l within 60-minutes post dose, followed by an exponential decay back towards resting levels by 240-minutes post dose.

In the feeding cohort (Fig.3.1B) there were main effects of treatment ( $P < 0.01$ ), time ( $P < 0.001$ ) and treatment x time ( $P < 0.001$ ). Plasma BHB levels across trial F30 were significantly lower than both trial F90 ( $P < 0.05$ ) and trial F4:1 ( $P < 0.05$ ) and across trials plasma BHB at time-points 15, 30, 45, 60, 75, 90 and 120-minutes was significantly elevated from baseline ( $P < 0.05$ ). Plasma BHB in trial F30 was significantly lower at

the 30, 45 and 60-minute time-points compared to trial F90 ( $P<0.05$ ) and at the 15, 30, 45, 60 ( $P<0.01$ ) and 75-minute ( $P<0.05$ ) time-points compared to trial F4:1.

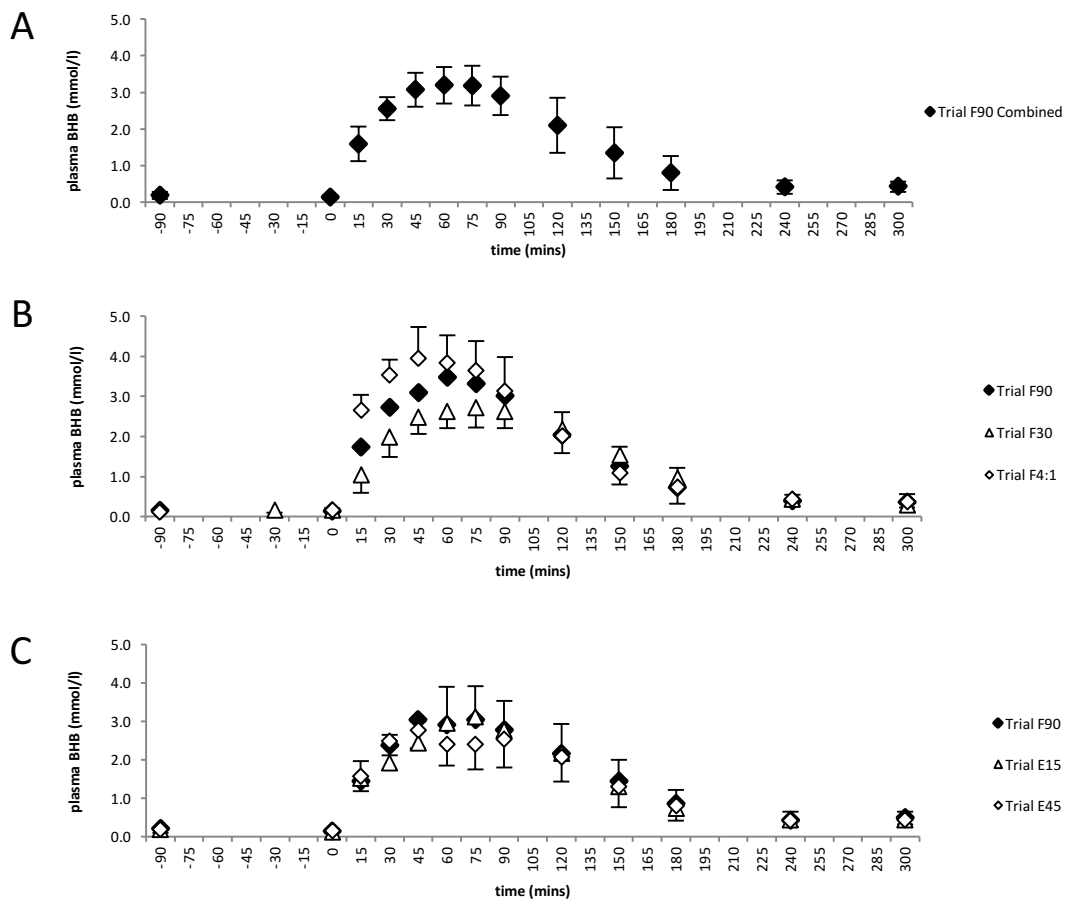


Figure 3.2. Time series plots of plasma betahydroxybutyrate (BHB) across trials. A (n=10): Trial F90 for all participants; B (n=5): Feeding trial cohort showing Trial F90 (90' Feed), Trial F30 (30' Feed) and Trial F4:1 (4:1 K:C Feed) with main effect of treatment ( $P<0.01$ ), time ( $P<0.001$ ) and treatment x time ( $P<0.001$ ); C (n=5): Exercise trial cohort showing Trial F90 (90' Feed), Trial E15 (15' Exercise) and Trial E45 (45' Exercise) with main effect of time ( $P<0.01$ ) only. All data are normalised to ketone ester dosing at time zero and presented as mean  $\pm$  SD.

In the exercise cohort (Fig.3.1C) there was a main effect of time only ( $P<0.01$ ) with time-points 15, 30, 45, 60, 75, 90 and 120-minutes all significantly elevated from baseline ( $P<0.05$ ).

	Feeding Cohort				Exercise Cohort		
	Trial F90 (All)	Trial F90	Trial F30	Trial F4:1	Trial F90	Trial E15	Trial E45
C <sub>max</sub> (mmol/l)	3.4 ± 0.5	3.5 ± 0.5	2.8 ± 0.4 <sup>+</sup>	4.1 ± 0.7 <sup>**</sup>	3.2 ± 0.4	3.2 ± 0.9	3.0 ± 0.4
T <sub>max</sub> (mins)	57.0 ± 13.8	63.0 ± 6.7	75.0 ± 10.6	51.0 ± 17.1	51.0 ± 17.1	69.0 ± 8.2	60.0 ± 28.1
t <sub>1/2</sub> (mins)	73.2 ± 17.3	75.8 ± 25.5	58.2 ± 10.4	62.0 ± 7.2	70.6 ± 3.1	66.3 ± 11.8	76.4 ± 13.2
AUC: 0-inf (mmol/l*min)	490.2 ± 108.9	486.7 ± 117.2	438.5 ± 83.5	536.2 ± 120.8	493.6 ± 113.6	461.1 ± 136.9	457.2 ± 109.2
AUC: 0-90 (mmol/l*min)		260.7 ± 33.6	201.9 ± 33.4 <sup>*</sup>	311.4 ± 49.3 <sup>*</sup>			
AUC: 15-45 (mmol/l*min)					81.3 ± 8.0	65.4 ± 18.4	78.6 ± 10.0
AUC: 45-75 (mmol/l*min)					89.1 ± 10.8	90.9 ± 25.2	72.3 ± 17.9

Table 3.1. Pharmacokinetic parameters (C<sub>max</sub> = peak concentration of BHB; T<sub>max</sub> = time to peak concentration of BHB; t<sub>1/2</sub> = half-life of BHB; AUC = area under the BHB curve across various time-points as indicated) compared by trial. Trial comparisons versus trial F90: <sup>+</sup> = P=0.07; <sup>\*</sup> = P<0.05; <sup>\*\*</sup> = P<0.01.

Analysis of variance of pharmacokinetic data (Table 3.1) indicated a main effect of treatment in the feeding cohort on peak BHB concentration (C<sub>max</sub>; P=0.003) and area under BHB curve in the 90-minutes post dose (AUC: 0-90; P=0.01). Compared to Trial F90, C<sub>max</sub> was significantly higher in Trial F4:1 (P<0.01) and tended to be lower in Trial F30 without reaching significance (P=0.07). AUC: 0-90 in Trial F30 was lower than Trial F90 (P<0.05) and in Trial F4:1 was higher than Trial F90 (P<0.05). There were no significant trial effects on time to peak BHB concentration (T<sub>max</sub>), BHB half life (t<sub>1/2</sub>) or overall area under the BHB curve (AUC: 0-inf). In the exercise cohort there were non-significant trends towards a lower area under the BHB curve during the exercise periods but no overall treatment effect was observed.

Urinalysis conducted at the end of each trial indicated average urinary ketone concentration of 0.28 ± 0.16g/l with average urinary excretion volumes of 893.7 ±

617.6ml, suggesting a negligible impact of urinary ketone disposal over the trial period.

## **DISCUSSION**

The availability of circulating ketone bodies following acute consumption of a bolus of dietary ketone ester is influenced by the timing of food intake prior to ketone ester consumption and the ratio of ketone ester to carbohydrate consumed, but does not appear to be significantly impacted by the provision and timing of warm up exercise after ketone ester consumption. These factors are important elements of preparation for sports performance (Hawley & Burke, 1997) and as such warrant careful consideration when adopting nutritional ketosis as an interventional strategy in the field.

The pharmacokinetics of the plasma betahydroxybutyrate (BHB) response to ketone ester consumption are characterised by a predictable rise to peak concentration after around one hour, followed by an exponential decay with clearance occurring after approximately four hours, in the absence of strenuous exercise. This response pattern provides a reliable platform for advice regarding the timing of ketone ester intake for sports performance with a timeframe of ~30-90-minutes post ketone ester dose appearing to provide an optimal window of moderate ketosis (Fig.3.1). Depending upon the utilisation of ketone bodies as an available exercising substrate, it may be necessary during prolonged exercise to provide top-up doses of ketone ester during the exercise period to sustain adequate ketone body supply.

Given the potential for gastric intolerance with exercise feeding strategies, and preliminary evidence that the consumption of ketone esters may result in gastrointestinal (GI) side effects (Clarke et al., 2012), the timing of ketone ester consumption needs to meet the competing demands of minimising gastric upset whilst maximising circulating ketone body availability during exercise. Consuming a meal within 30-minutes of ketone ester consumption in the current study moderately suppressed ketone body availability in the first 90-minutes after dosing (Fig.3.1B). A standardised breakfast was provided to replicate the typical practice of athletes in the field and to mitigate for the known effects of breakfast consumption on post-prandial metabolism (Gonzalez, Veasey, Rumbold, & Stevenson, 2013). Whilst a favourable release profile was seen when ketone ester was delivered 90-minutes after breakfast this may not be practical in all sports settings where a number of factors will influence pre-exercise feeding strategy (Ormsbee, Bach, & Baur, 2014).

Reducing the extent of carbohydrate co-dosing with ketone ester consumption resulted here in a more rapid release profile with a higher peak BHB concentration (Table 3.1). Whilst this may be desirable in some circumstances it is likely that most practical ketone ester dosing strategies will favour an optimal carbohydrate co-dosing regimen to maximise exercising substrate supply (Jeukendrup, 2004). Although not examined in the current study, it also remains possible that the rapid pharmacokinetic profile seen here with low carbohydrate delivery could exaggerate the gastro-intestinal side effects of ketone ester consumption during exercise.

Ketone body availability was not significantly compromised in the current study by a short bout of warm-up exercise typical of the preparation athletes would do before sports performance (Fig.3.1C). Were a significantly more vigorous warm-up employed, or a more prolonged period of exercise supported, it is likely that the dosing regimen described here would need to be revised to sustain the provision of ketotic substrate.

### **Conclusion**

To provide a viable strategy for athletes seeking to optimise fuel supply during sports performance any feeding strategy must be deployable within the constraints of the competition environment. Such considerations are of critical importance in exploring the translational value of promising ergogenic interventions evidenced in a laboratory context for utility in the field. The effects described here of a slowing of the accumulation of circulating ketone bodies when ketone ester dosing occurs sooner after a meal and a more rapid accumulation of circulating ketone bodies with a drink formulation lower in carbohydrate concentration are critical factors in the selection of dosing regimens for optimal ergogenic benefit of nutritional ketosis.



## Chapter 4: The Effect of Low-Dose Ketone Ester Consumption on Exercise Metabolism and Long Duration Cycling Performance

### ABSTRACT

A novel ketone ester drink has been developed that shows benefit to exercise energetics and endurance performance in trained athletes (Cox et al., 2016). However, ketone ester consumption comes with a significant side effect profile of gastro-intestinal (GI) symptoms that could inhibit the ergogenic potential of nutritional ketosis. The present study aimed to determine, in twelve well trained men ( $VO_{2max}$ :  $64.4 \pm 6.1$  ml/kg/min), whether consumption of ketone ester (KET) at a lower dose than previously described (6mg/min/kg-body-mass) could improve exercising metabolism and cycling performance during a mixed-demand 100-min effort followed by a 20km time trial (TT), when compared to a calorie-matched carbohydrate drink (CHO). KET consumption lowered exercising plasma NEFA ( $P < 0.05$ ) and glycerol ( $P < 0.05$ ) and negated the drop in RER across the 100-min effort seen with CHO ( $P < 0.05$ ), but had no impact on plasma glucose or lactate. Despite a low GI symptom profile KET had no impact upon TT performance (KET v CHO:  $1731.3 \pm 113.0$ s v  $1736.9 \pm 114.2$ s) although there was a small trial order effect. Altered perceptions of effort ( $P < 0.05$ ) and affect ( $P < 0.05$ ) during the 100-min effort correlated with the change in TT performance. In conclusion, low dose ketone ester consumption did not improve long duration cycling performance despite low GI symptoms and altered indices of fat metabolism.

## INTRODUCTION

A novel dietary ketone monoester has recently been developed that can rapidly induce a moderate ketosis and in doing so offer a novel alternate source of energetic substrate that would not normally be available during exercise in a eucaloric feeding state (Clarke et al., 2012). Ketone bodies are excellent fuel sources with rapid availability and excellent mitochondrial efficiency. Acute ingestion of this ketone ester has been shown (unpublished data from our group) to exert a positive impact on athletic performance in high performance athletes, both in rowers during a 30' rowing ergometer distance trial and in rugby players during an 84' rugby game simulation test (BURST). However, the prevalence of gastrointestinal side effects during moderate ketone ester intake is high (Clarke et al., 2012), with the potential to dampen any ergogenic benefit that might ensue from consumption. The effect of nutritional ketosis on prolonged endurance performance is currently unknown but significant potential exists for ergogenic benefit if gastrointestinal side effects can be managed within tolerable limits.

The performance of prolonged exercise requires a sustained supply of adequate fuel for muscular work in the face of finite endogenous fuel stores and a limited capacity to uptake and utilise exogenous dietary fuel (Bosch, Dennis, & Noakes, 1994). A large number of studies have shown that long duration exercise performance can be influenced by a variety of acute feeding strategies that alter exogenous substrate supply (e.g. Hargreaves, Hawley, & Jeukendrup, 2004; Mitchell et al., 1989). In particular sustaining high-intensity exercise for durations in excess of 90 minutes has

been shown to benefit from the supply of dietary carbohydrate both prior to and during the exercise bout (Jeukendrup, 2004). However, where exercise is of a shorter duration and/or intermittent in nature the evidence is far more equivocal, particularly for performance in a glycogen replete state (Coombes & Hamilton, 2000). Due to the varied complications of prior feeding, the training status of participants and the nature of the task imposed, the defining features of situation and treatment that lead to benefit remain somewhat elusive.

In the search for explanatory factors in defining this performance variance the concept of pacing for optimal performance has emerged (Tucker & Noakes, 2009). It has been shown that pace selection during an exercise performance task is dynamically regulated to ensure optimal performance output and that this regulation can be influenced by feed forward factors relating to motivational drive and feedback factors relating to available resources. In the context of fuel status, manipulation of muscle glycogen stores can alter pace selection (Rauch, St Clair Gibson, Lambert, & Noakes, 2005) and even the unfulfilled promise of further exogenous carbohydrate delivery, via carbohydrate mouth rinsing, can mobilise the same response (Beaven, Maulder, Pooley, Kilduff, & Cook, 2013; Carter et al., 2004). Major differences in motivational state have been reported for highly trained athletes in comparison to recreationally active people or less well trained competitive athletes indicating that from a pacing perspective observations regarding feeding intervention in one group may not meaningfully translate to another. Whether nutritional ketosis can add a further dimension to this performance dynamic is an intriguing prospect.

This study aimed to determine whether supplemental ketosis via ketone ester feeding, at a lower dose than utilised previously, could improve long duration cycling performance without significant gastrointestinal side effects in highly trained endurance athletes compared to current best practice macronutrient feeding employing multiple transportable carbohydrates (Currell & Jeukendrup, 2008). An intermittent exercise model consisting of a pre-load period followed by a time trial was chosen to simulate the non-steady state nature of a typical cycling endurance event.

## **METHODS**

### **Participants**

Twelve healthy men participated in this study (age  $29.8 \pm 6.5$  yr; height  $183.4 \pm 5.5$  cm; body mass (BM)  $74.9 \pm 6.9$  kg). All participants completed a self-report training questionnaire and were categorised as 'well-trained' or 'top-level competitive' cyclists or triathletes with a training history of  $12.9 \pm 7.1$  years and an average weekly training volume of  $10.3 \pm 3.0$  hours. The study received approval from the University of Bath Research Ethics Approval Committee for Health and each participant was provided with a written and verbal briefing regarding the nature of the testing, and provided written, informed consent prior to participation.

## Experimental Design

This study followed a double-blinded, randomised cross-over design. Participants attended the laboratory on three occasions each separated by 5-8 days. On the first preliminary trial day all participants performed an incremental cycle exercise test followed, after one hour of recovery, by a 20km time trial with the aim of determining participant work capacity and aerobic fitness whilst also achieving familiarisation with the test procedure for subsequent trials. On the following two main trial days a 20km time trial was performed following the completion of a 100-minute submaximal mixed intensity cycling pre-load. Participants were randomly assigned drinks containing either 1.2g/min carbohydrate + 6mg/min/kgBW ketone ester (KET) or 1.2g/min carbohydrate + further carbohydrate at the calorific equivalent of the ketone ester arm (CHO). Carbohydrate was provided in accordance with current sports nutrition guidelines in the form of glucose and fructose in the ratio 2:1 (Currell & Jeukendrup, 2008). Drinks were blinded by colour and flavour matching and were also matched for volume by the addition of water. Further water was provided ad-libitum during the trial and water intake was recorded. All exercise was conducted on an electronically braked, stationary cycle ergometer (Schoberer Rad Messtechnik, SRM, Germany) under controlled laboratory conditions at a moderate ambient heat load of 25°C and 50% humidity.

## **Preliminary Visit**

### *Incremental Exercise Test*

An exhaustive incremental exercise test was performed on the cycle ergometer (SRM) for the determination of maximal oxygen uptake ( $VO_{2max}$ ) and maximum power output ( $W_{max}$ ). Expired gases were collected in the final minute of exercise using a Douglas bag and subsequently analysed for volume of air expired using a dry gas meter (Harvard Apparatus, UK), temperature of expired gases via a digital thermometer (model C, Edale Instruments, UK) and fractional concentrations of  $O_2$  and  $CO_2$  using paramagnetic and infra-red methods, respectively (Servoflex MiniMP, Servomex, UK). These analysers were calibrated prior to each test with gases of known composition and volume within the physiological range, as certified by prior gravimetric analysis (British Oxygen Company, UK), and measured values were used to determine the maximal rate of oxygen consumption ( $VO_{2max}$ ). Starting load was self-selected based on warm up intensity and the exercise protocol consisted of 30W increments every three minutes for fifteen minutes followed by 20W increments per minute until exhaustion. Power output during the final minute was averaged to represent work capacity ( $W_{max}$ ). The results of this test were used to determine the workload for the pre-load phase on the main trial visits.

### *Familiarisation Time Trial*

All participants were required to complete a preliminary performance of the 20km time trial, following one hour of recovery from the incremental test, in order to

ensure that they were familiar with the pacing requirements and experimental set up. During the time trial participants were provided with real time feedback only on current distance covered and instructed to cover the race distance as quickly as possible. Standardised phrases of encouragement were provided at set intervals of elapsed distance (5km, 10km, 15km, 19km, 19.5km, 19.9km) during the time trial. No music was played and no intrusive physiological measures were collected until after the trial was completed. Each participant's chosen bicycle geometry was recorded (vertical and horizontal saddle position and vertical and horizontal handlebar position) and repeated on subsequent visits. Participants provided their own cycling pedals and shoes with the stipulation that the same equipment be used across all trials.

## **Main Performance Trial Visits**

### *Experimental Protocol*

For each main trial visit participants were instructed to consume a standardised meal the evening before and a standardised breakfast on the morning of the trial before arrival at the laboratory. Upon arrival baseline blood samples were collected and participants were fitted with a body sensor network garment (Hidalgo Equivital) for the continuous measurement of heart rate, breathing rate, skin temperature and core temperature (via telemetry from a thermometric pill ingested four hours before the trial; Philips VitalSense). Participants then completed a 100-minute pre-load period, consisting of five continuous 20-minute bouts of cycling that alternated



between blocks of constant load at the power output that elicited 60% of  $VO_{2max}$  on the incremental exercise test and blocks of intermittent load (alternating 150-second work-periods at the power outputs that elicited 40% and 80% of  $VO_{2max}$ ) followed, after a 5-minute break, by a 20km time trial adopting the same procedures as the familiarisation visit (Figure 4.1). Experimental drinks were separated by volume and consumed as a 40% starting bolus delivered 45 minutes prior to the onset of exercise, followed by six 10% doses delivered at 20 minute intervals during exercise.

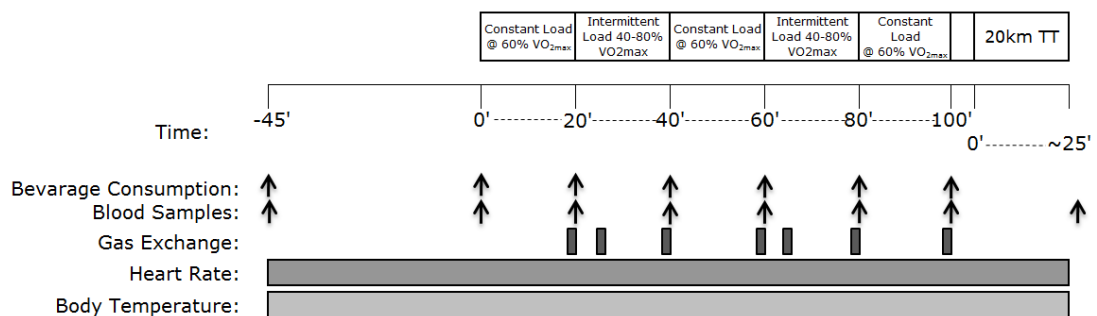


Figure 4.1: Main Trial: Exercise protocol, timing of ketone ester dosing, timing of blood sampling and gas exchange, heart rate and body temperature measurement.

### Experimental Measurement Methods

Capillary blood samples (0.5ml) were collected from an earlobe into EDTA sample buckets (Microvette 500, Sarstedt, Nümbrecht, Germany). Samples were taken at baseline and then immediately prior to and each 20 minutes block throughout the exercise period. A final sample was collected after completion of the time trial. Samples were analysed immediately for blood ketone concentration using a whole blood microfluidic strip method (Optium Xceed) by an experimenter who was not

present at the performance of the time trials. The remaining blood was centrifuged and the plasma fraction retained (thereby rendering the sample acellular) and stored at  $-80^{\circ}\text{C}$  for later analysis of glucose, lactate, glycerol and free fatty acids. Blood metabolites were analysed by spectrophotometry using commercially available analysis kits (RX Daytona, Randox, Crumlin, UK).

At baseline, after each block of intermittent load and after the time trial an additional earlobe capillary blood sample (100ul) was collected for blood gas analysis. Capillary samples were drawn into pre-treated heparin glass capillaries (Clinitubes, Radiometer, Brønshøj, Denmark) by capillary action from a blub of fresh blood formed at the base of the earlobe. Samples were agitated by hand for 30 seconds and then immediately presented to an automated blood gas analyser (ABL 70, Radiometer, Brønshøj, Denmark) for the measurement of pH,  $\text{pO}_2$  and  $\text{pCO}_2$  and the derivation of blood bicarbonate concentration and base excess (Burnett & Noonan, 1974).

Expired gases were collected in a Douglas bag for five-minutes at baseline and then for one minute periods in the 20<sup>th</sup>, 25<sup>th</sup>, 40<sup>th</sup>, 60<sup>th</sup>, 65<sup>th</sup>, 80<sup>th</sup> and 100<sup>th</sup> minute of the pre-load exercise period. Expired gas samples were analysed, following the procedures described in the incremental exercise test, for the determination of ventilation, oxygen consumption, carbon dioxide production and respiratory exchange ratio. Throughout the trial body temperature was measured by an ingested

core temperature pill (Philips VitalSense) via telemetry to an ambulatory data logger (Hidalgo Equivital), which also recorded axillary skin temperature via a surface thermistor.

During the trial participants were asked to provide regular subjective ratings of their perceived exertion levels, affective state and gastrointestinal comfort (see General Methods), whilst perceptions of drink pleasantness were recorded at each ingestion point. All participants completed an exit questionnaire (Appendix 6) after their final trial to determine whether they could ascertain, whilst still blinded to the trial order, which drink was the ketone ester product and on which trial they had performed better in the time trial.

### **Statistical Analysis**

A paired two-tailed t test was used to identify differences between treatments, and a two-way (treatment x time) general liner model for repeated measures was employed for all serial measures, with the Greenhouse-Geisser correction applied where assumptions of sphericity were violated. Main effects were isolated by pairwise multiple comparisons using the Bonferroni method. Treatment effects on time trial performance were compared to overall time trial performance using Bland Altman plots and compared with treatment effects for independent variables (objective and subjective data) by correlation analysis. All statistical analysis was

conducted using IBM SPSS Statistics (Release 20.0.0, IBM, USA) and all data are presented as mean  $\pm$  SD. Statistical significance was set at the level of  $P < 0.05$ .

## **RESULTS**

### *Participant Fitness and Training History*

Participant fitness (Table 4.1A) was demonstrated by a group average maximum minute power of  $400.4 \pm 34.6$ W and body weight corrected maximal oxygen consumption of  $64.4 \pm 6.1$ ml/kg/min. All participants were self-rated as either a well-trained ( $n=9$ ) or a top-level/competitive ( $n=3$ ) athlete and all reported being competitively active members of a sports club. Nine participants held a British Cycling racing license (Cat 3:  $n=4$ ; Cat 2:  $n=4$ ; Cat 1:  $n=1$ ) and three participants competed in triathlon. Training history data (Table 4.1B) indicated that the group on average had been in exercise training for  $12.9 \pm 7.1$  years and currently completed an average weekly training volume of  $10.3 \pm 3.0$  hours.

<b>A</b>		Age	Height	Weight		
Ave		29.8	183.4	74.9		
SD		± 6.5	± 5.5	± 6.9		
		1' W <sub>max</sub> (W)	VO <sub>2max</sub> (L/min) (ml/kg/min)		20km TT (mm:ss.0) (W) (W/kg)	
Ave		400.4	4.8	64.4	27:00.7	302.7
SD		± 34.6	± 0.2	± 6.1	± 01:12.6	± 30.2
<b>B</b>		Exercise History (yrs)	Weekly Exercise Sessions	Weekly Cycling Sessions	Weekly Exercise Volume (hrs)	
Ave		12.9	6.4	5.4	10.3	
SD		7.1	1.8	2.2	3.0	

Table 4.1. Participant characteristics (n=12 male cyclists). A: Participant demographics (age, height and weight), one minute work capacity (1' W<sub>max</sub>), aerobic capacity (absolute and relative VO<sub>2max</sub>) and 20km time trial performance (time to completion, average power output and power output per kg body weight). B: Participant training history (exercise history, weekly overall and cycling sessions and weekly exercise volume). All data are mean ± SD.

### *Time Trial Performance*

There was no significant treatment effect on time trial performance for time to completion (KET v CHO: 1731.3 ± 113.0s v 1736.9 ± 114.2s; P=N.S.) or average power output (260.1 ± 45.1W v 258.7 ± 43.5W; P=N.S.). However, a trial order effect was present for time to completion (Trial B v Trial A: 1716.0 ± 113.2s v 1752.2 ± 111.0s; P<0.05; Fig 4.2) and average power output (265.3 ± 45.8W v 253.5 ± 41.8W; P<0.05) with the second trial (Trial B) faster than the first (Trial A).

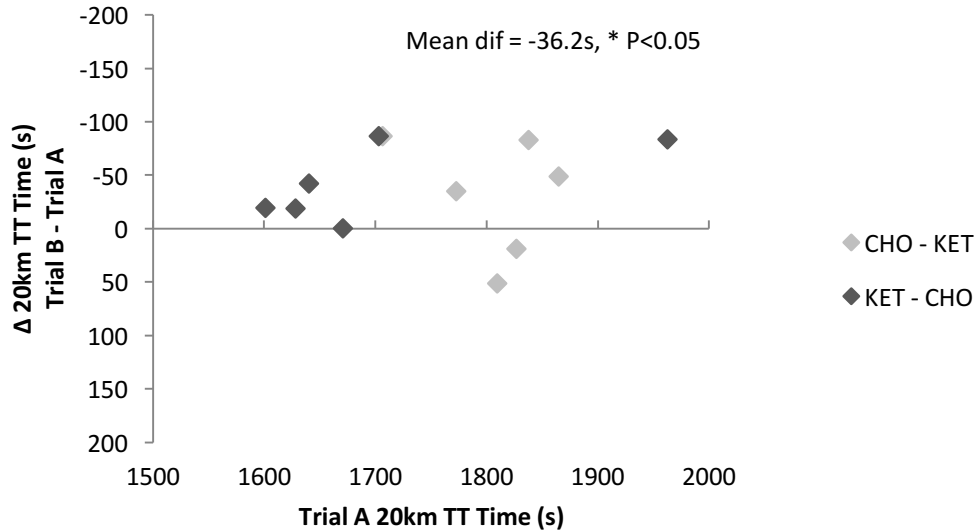


Figure 4.2. Difference between trials (B-A) in time to completion of the 20km time trial versus time taken to complete the first main trial (Trial A; n=12 male cyclists). Closed symbols represent participants who received the ketone ester (KET) treatment in trial B and open symbols represent participants who received the ketone ester (KET) treatment in trial A. Trial B v Trial A: \*P<0.05.

#### *Blood Metabolite Analysis*

There were no main effects of treatment on plasma glucose or lactate but main treatment effects were seen for plasma NEFA and glycerol (Fig 4.3) with the ketone ester treatment suppressing both responses compared to carbohydrate. Trial order had no significant effect on any blood metabolite parameter.

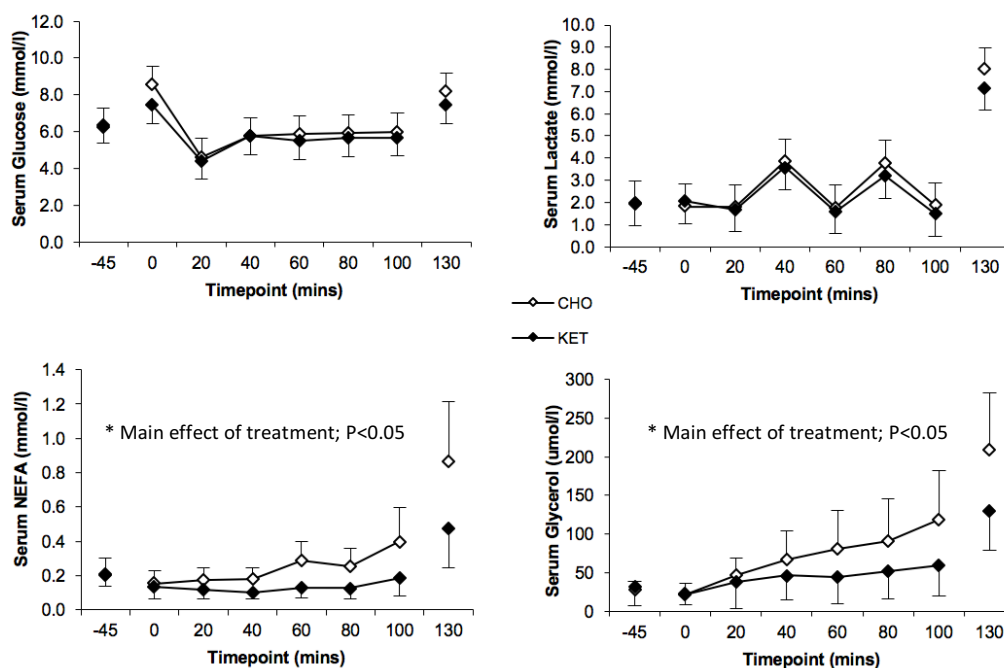


Figure 4.3: Blood glucose, lactate, NEFA and glycerol responses to the exercise protocol (n=12 male cyclists): Closed symbols represent the ketone ester (KET) trial and open symbols represent the carbohydrate (CHO) trial. Treatment comparisons: \* P < 0.05.

#### Blood Ketone Bodies and Gas Analysis

Ketone ester treatment resulted in a rapid and sustained elevation of blood ketone body concentration throughout exercise resulting in average blood ketone body concentration in the ketone ester (KET) trial of  $1.29 \pm 0.39$  mmol/l. There were also main effects of treatment for pH and bicarbonate with ketone ester treatment resulting in a lowering of both pH and blood bicarbonate concentration at both mid-exercise timepoints (Fig 4.4). Trial order did not alter blood gas status. Blood SpO<sub>2</sub> levels averaged 94-96% indicating good quality signal throughout.



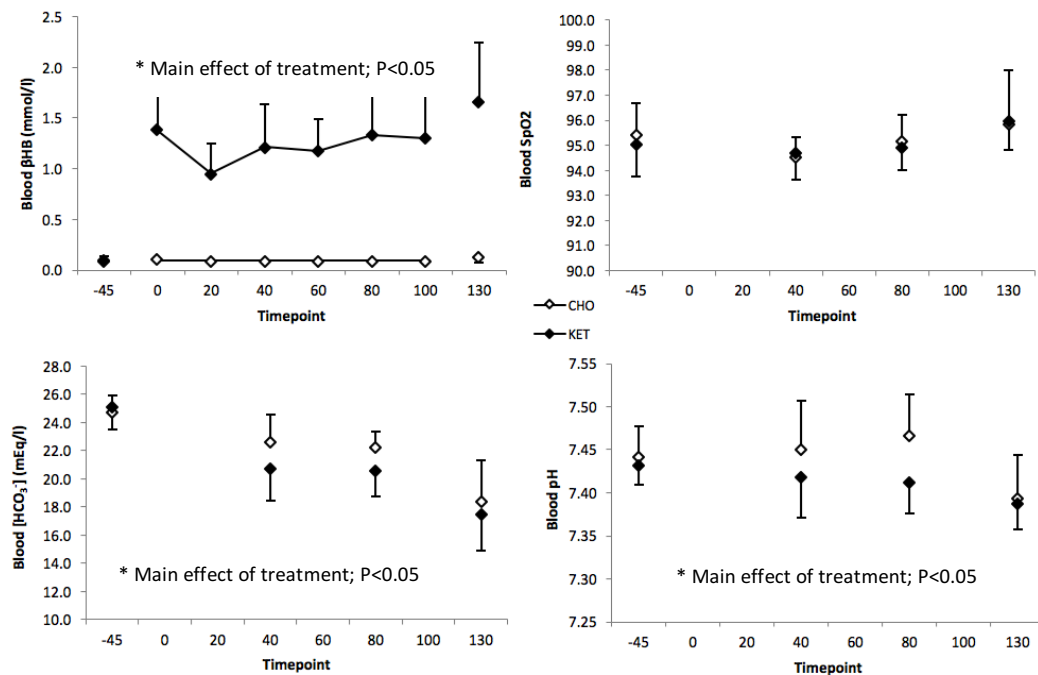


Figure 4.4. Blood ketone body (BHB), oxygen saturation (SpO<sub>2</sub>), bicarbonate (HCO<sub>3</sub><sup>-</sup>) and pH responses to the exercise protocol (n=12 male cyclists): Closed symbols represent the ketone ester (KET) trial and open symbols represent the carbohydrate (CHO) trial. Treatment comparisons: \* P < 0.05.

### Respired Gas Analysis

Energy expenditure, as measured by percentage of VO<sub>2max</sub>, was on target for the pre-load period averaging 60.3 ± 4.1% and 78.1 ± 4.6% across all trials at the pre-defined workloads. There were no differences in ventilation, VO<sub>2</sub> or VCO<sub>2</sub> with treatment. However, the change in RER across the submaximal exercise period was significantly attenuated by the ketone ester treatment (KET v CHO: 0.00 ± 0.02 v -0.03 ± 0.04; P < 0.05) indicating a differential shift in substrate selection.

### Body Temperature and Fluid Balance

Ambient temperature (24.6 ± 0.3 v 24.6 ± 0.4 °C) and relative humidity (48.9 ± 6.1 v 47.8 ± 5.3 %) were similar between trials. Only 7/12 core temperature recordings

were of sufficient quality for inclusion in the study. As a result, no analysis was performed on this data. Several of the unacceptable recordings suffered for temperature fluctuation with fluid intake, indicating that insufficient gastric transit had taken place. However, one pill could not be detected at all, suggesting that it had been evacuated prior to the onset of the study. Such large variations in gastric transit time make reliable data capture unpredictable with the result that a significantly larger study cohort would have been required to sufficiently power a statistical analysis.

Significant rises in skin temperature were observed across the pre-load period in all trials and trended towards being higher in the carbohydrate (CHO) trial compared to the ketone ester (KET) trial but this did not reach significance (KET v CHO:  $2.6 \pm 0.8^{\circ}\text{C}$  v  $3.2 \pm 1.0^{\circ}\text{C}$ ;  $P=0.08$ ). There were no differences in body weight change or calculated sweat loss across the trials either by treatment or order but an order effect was seen for volitional water intake, which was lower in the second trial (Trial B v Trial A:  $1002 \pm 310\text{ml}$  v  $1181 \pm 441\text{ml}$ ;  $P<0.05$ ).

### *Subjective Ratings*

Participants rated their exertion during the pre-load period on average as fairly light (@ 60% load) to fairly heavy (@80% load) with ratings of feeling starting on average as good and drifting down to fairly good as the trial progressed. Neither treatment

nor trial order influenced perceived exertion or affect during the pre-load period or following the time trial.

The ketone ester (KET) trial drink was rated as less pleasant (moderately to strongly unpleasant) than the carbohydrate (CHO) trial drink (neutral to moderately unpleasant), although there were a wide range of individual ratings (KET v CHO:  $40.4 \pm 7.3$  v  $49.7 \pm 6.6$ ;  $P < 0.001$ ).

Mild to moderate GI symptoms presented on both treatments in a minority of participants but with no clear effect of treatment or trial order (Fig 4.5).

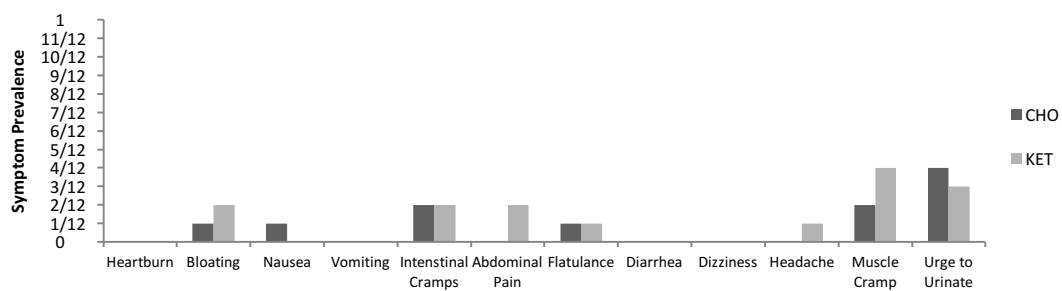


Figure 4.5. Prevalence of mild to moderate GI symptoms during the exercise protocol: number of subjects presenting with a given symptom; KET v CHO (n=12 male cyclists).

#### *Time Trial Performance Correlates*

The difference in time trial performance between treatments correlated with the difference in pre-load affect ( $P=0.02$ ) and pre-load RPE ( $P=0.02$ ) and trended towards a relationship with the difference in GI symptoms ( $P=0.09$ ; Fig 4.6).

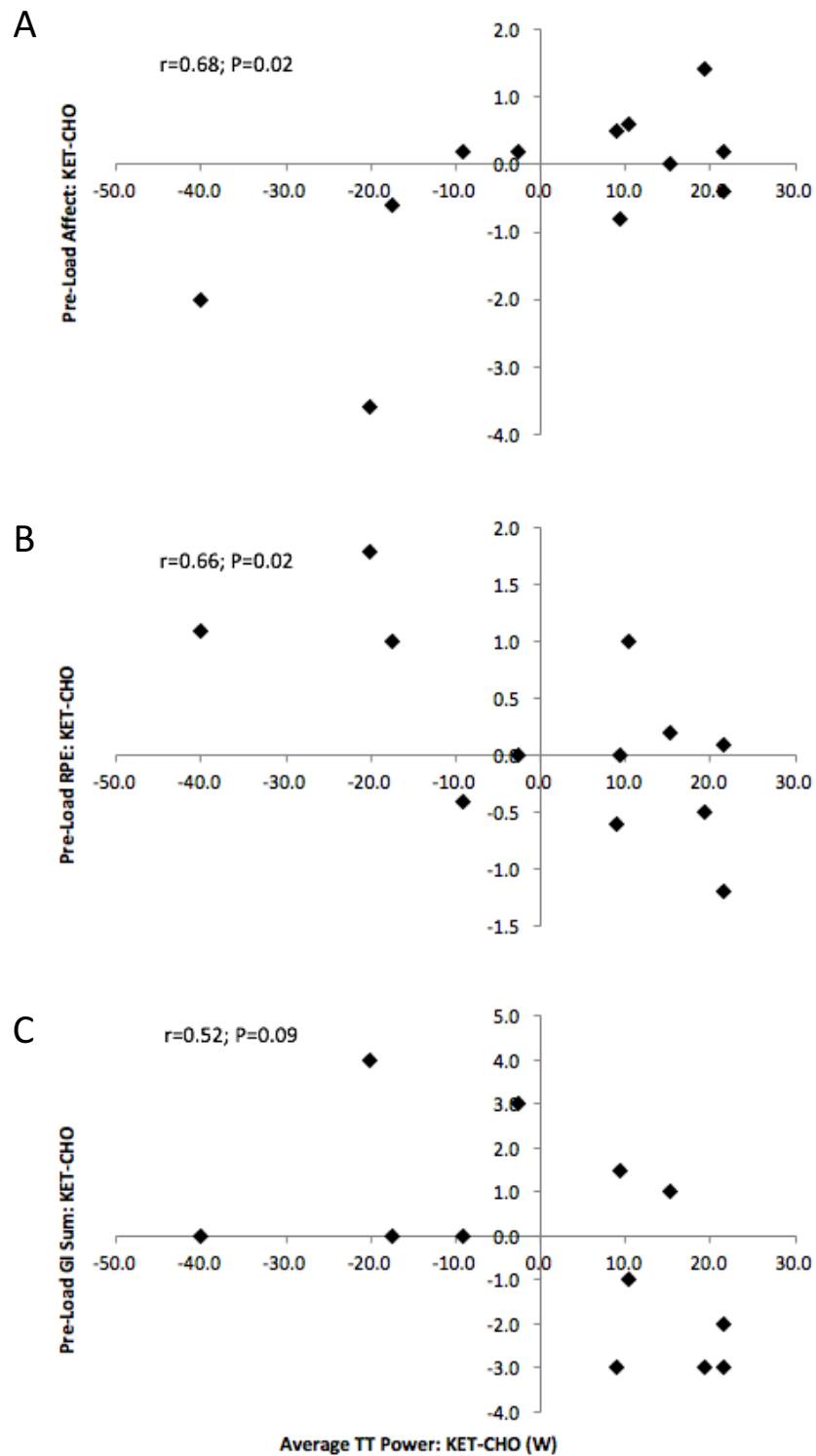


Figure 4.6. Scatter plots and statistical correlations for treatment differences (KET-CHO) in average time trial (TT) power versus subjective ratings during pre-load (n=12 male cyclists): A. Average pre-load affect ( $r=0.68$ ;  $P=0.02$ ); B. Average pre-load RPE ( $r=0.66$ ;  $P=0.02$ ); C. Sum of pre-load gastrointestinal symptoms ( $r=0.52$ ;  $P=0.09$ ).

The difference in time trial performance between treatments was also related to differences in water intake with lower water intake rates associated with better time trial performance ( $r=-0.66$ ;  $P=0.02$ ).

#### *Exit Questionnaire*

Participants were unable to predict which of the drinks was the active treatment (6 guessed correctly, 5 guessed incorrectly and one couldn't discern a difference) or on which time trial they performed better (5 guessed correctly and 7 guessed incorrectly).

## **DISCUSSION**

The main finding of this study was that low dose ketone ester consumption had no impact on the performance of a 20km time trial following a 100-minute period of mixed-intensity submaximal cycling. This observation was confounded by a trial order effect on time trial performance that occurred despite the presence of a familiarisation trial. Had a more extensive familiarisation reduced the CV to half of that observed here, three times as many participants would have been required to assess the small treatment effect observed of 0.7% with sufficient statistical power.

A lower dosage of ketone ester was chosen for this study in an attempt to minimise the gastrointestinal side effects associated with ester consumption. Whilst this

dosing regimen was successful in moderating GI symptom occurrence to a level comparable to the carbohydrate fed in the control treatment, this may have come at the expense of an ergogenic effect similar to that seen in previous trials at a higher dose (unpublished data from our group). The dose delivered was sufficient to induce a sustained ketosis but at a lower level ( $1.29 \pm 0.39$ mmol/l) than that achieved in previous trials with a higher dosing regimen (2-3mmol/l). Future trials should consider whether there is a lower threshold of ketosis required to confer ergogenic benefit in a given exercise model.

The ketosis induced by ketone ester consumption in this study was sufficient to have a significant impact on circulating lipid metabolites during the pre-load exercise period suggesting that exercising substrate selection was altered by ketone ester treatment. The accumulation over time of blood NEFA and Glycerol levels in the carbohydrate (CHO) trial was consistent with previously published observations of a switch in substrate selection towards an increase in fat oxidation and decrease in carbohydrate oxidation over time with prolonged exercise (J a Romijn, Coyle, Sidossis, Rosenblatt, & Wolfe, 2000). This view was further supported by a drop in RER across the pre-load exercise period indicative of such a shift in substrate selection in the carbohydrate (CHO) trial. However, following ketone ester treatment there was a significant attenuation of the rise in both plasma NEFA and glycerol and a concomitant negation of the drop in RER with submaximal exercise, indicating a likely shift away from increasing reliance on lipid oxidation as exercise progressed. Quantitative evaluation of changes in substrate oxidation from respiratory gas exchange data is not possible in the presence of significant levels of ketone bodies

since they are present in a racemic mix of betahydroxybutyrate and acetoacetate and each of these compounds has an RQ somewhere between that of fat and carbohydrate, rendering such estimation methods invalid. It would be of significant value to quantify rates of substrate oxidation in the presence of ketosis during exercise by conducting isotopic tracer studies.

The low level ketosis seen here was not sufficient to significantly alter blood glucose or lactate concentrations during exercise. This finding is in contrast to previous studies with higher ketone ester doses and may point towards a potential mechanism of ergogenic benefit for ketone ester treatment in those trials (Cox et al., 2016). The exercise protocol chosen here resulted in sustained work output at a lower level to that seen in previous studies resulting in significantly lower levels of circulating lactate. Whilst the lower dose ketosis itself cannot be ruled out as a factor in the lack of an ergogenic effect in the current trial, it remains possible that a higher level of sustained workload demand may be required to expose an impact of ketosis on lactate metabolism that is sufficient to confer functional benefit.

Ketone ester treatment did result in transient changes in blood gas status with more rapid degradation of bicarbonate stores and lowering of pH evident during the pre-load period. These changes most likely relate to the increase in acid load associated with ketone body loading. They were no longer present at peak exercise following the time trial. Whilst an increase in submaximal acid load may appear undesirable during exercise, it could confer an advantage during certain types of work where sufficient buffering capacity exists, since monocarboxylates appear to be preferred as a rapidly available substrate during periods of high energetic demand (George A



Brooks, 2009). Indeed, recently attempts have been made to positively impact exercise performance by raising monocarboxylate levels in the form of the consumption of exogenous lactic acid (Brooks, personal communication). It would be worthwhile to explore further the role of ketone ester consumption in elevating the monocarboxylate pool to provide rapidly available substrate during sustained high intensity exercise.

The trial order effect observed on time trial performance may have resulted from insufficient familiarisation since participants were only familiarised to the time trial, following an incremental exercise test, and not the pre-load section of the protocol. That differences in volitional water intake related to differences in time trial performance across trials may be indicative of this and perhaps point towards an acclimation to the moderate thermal load with repeat trialling as a moderator of performance in the current trial (Guy, Deakin, Edwards, Miller, & Pyne, 2015).

However, there were no differences between the first and second trials in skin temperature, heart rate, blood metabolites or any gas exchange variable. Irrespective of treatment an improvement in time trial performance was associated with lower levels of perceived exertion and higher levels of affect during the pre-load exercise period. Given the highly subjective nature of pacing for volitional performance and the absence of any objective performance feedback during the time trials these subjective relationships are unsurprising but likely indicate some of the common factors which lead to natural variance in repeat performance of this type of exercise task in other settings (Sharma, Elliott, & Bentley, 2015). That the likely changes in substrate selection were not sufficient to impact perceptual

wellbeing may have been a key factor in the failure of the ketone ester to impact performance.

It is possible that perceived differences in the pleasantness of the trial drinks impacted individual participant's motivation to perform. However, the inability of participants to predict which drink was which and more importantly what their actual level of performance was between the two trials makes this an unlikely prospect. There was a non-significant trend for differences in GI symptoms to predict time trial performance, regardless of which drink provoked more symptoms for a given participant, however there was no overall difference in symptom appearance between treatments. Whilst the lower dosing used in the current trial may therefore have been sufficient to contain the excesses of symptom appearance reported previously, they were also insufficient to induce ergogenic benefit. It appears likely therefore that athletes seeking ergogenic gain from ketone ester consumption either need to be relatively tolerant to the drink or perhaps hardy enough to cope with its effects without derailing performance. There is evidence that more successful athletes in real world endurance events tolerate higher levels of GI symptoms in order to gain the energetic benefits of additional feeding during performance (Jeukendrup & Killer, 2011). This level of motivation may not have been adequately assessed in the laboratory setting of the current study where participants performed in isolation.

## **Conclusion**

Low dose ketone ester consumption was capable of altering substrate metabolism during long duration cycling, by dampening exercise induced elevations in serum NEFA and glycerol, but did not have any impact at the dose delivered on 20km cycling time trial performance following an extensive pre-fatigue protocol. Future studies should examine the dose response to ketone ester consumption in relation to its impact upon substrate selection at different intensities and for different durations of exercise as well as exploring the subjective response to feeding as a potential mitigating factor for performance impact.

## Chapter 5: The Androgenic Response to Repeat Sprint Exercise in Healthy Young Men

### ABSTRACT

Dihydrotestosterone (DHT) exerts both functional and signalling effects extending beyond the effects of testosterone in rodent skeletal muscle. As a primer for investigating the role of DHT in human skeletal muscle function, this study aimed to determine whether circulating DHT is acutely elevated in men following a bout of repeat sprint exercise and to establish the importance of training status and sprint performance to this response. Fourteen healthy active young men ( $VO_{2max}$   $61.0 \pm 8.1$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) performed a bout of repeat sprint cycle exercise at a target workload based on an incremental work-rate maximum (10x30s @ 150%  $W_{max}$  with 90-s recovery). Venous blood samples were collected pre-, 5 minutes post- and 60-minutes post-exercise. 5-minutes post-exercise there were significant elevations in total testosterone (TT;  $P < 0.001$ ), free testosterone (FT;  $P < 0.001$ ) and DHT ( $P = 0.004$ ), which returned to baseline after one hour. Changes in DHT with exercise (5' Post-Pre) correlated significantly with changes in TT ( $r = 0.870$ ;  $P < 0.001$ ) and FT ( $r = 0.914$ ;  $P < 0.001$ ). Sprinting cadence correlated with changes in FT ( $r = 0.697$ ;  $P = 0.006$ ), DHT ( $r = 0.625$ ;  $P = 0.017$ ) and TT ( $r = 0.603$ ;  $P = 0.022$ ) and habitual training volume correlated with the change in TT ( $r = 0.569$ ,  $P = 0.034$ ). In conclusion, our data demonstrates that DHT is acutely elevated following sprint cycle exercise and that this response is influenced by cycling cadence. The importance of DHT in the context of exercise training and sports performance remains to be determined.

## INTRODUCTION

Exercise is known to trigger acute elevations in circulating androgens, with responses that are dependent upon historical training status and workout design (Crewther et al 2006; Stokes et al 2012). These hormonal responses have been implicated both in the execution of acute workout performance and in the accrual of adaptive training gains (Crewther et al 2011). A variety of mechanisms have been proposed to support these actions, including the activation of cell signalling pathways promoting the mobilisation of energy reserves via GLUT4 (Sato et al 2008) and the accretion of protein for skeletal muscle hypertrophy (Ferrando et al 2002) via mTor (Wu et al 2010), modulation of the excitability of neuro-motor units (Bonifazi et al 2004) and more complex influences on behavioural motivation (Archer 2006) and cognition (Aleman et al 2004). However, the precise nature of the interaction between hormonal response and functional outcome in a given exercise setting remains poorly understood. In particular the reliance upon testosterone as a ubiquitous marker of the androgen system has come under scrutiny with recent attention widening to include another bioactive androgen, dihydrotestosterone (DHT) (Yarrow, McCoy & Borst 2012).

DHT is considered the terminal active product of androgen biosynthesis, being produced via both testosterone dependent and independent pathways (Yarrow, McCoy & Borst 2012). Compared to testosterone, DHT has greater affinity to the androgenic receptor (Bauer et al 2000). DHT has been described as an autocrine/paracrine hormone since it is formed in local tissue by the irreversible 5- $\alpha$

reduction of testosterone or androstenedione and is locally regulated in its action by an enzymatically controlled, reduction/oxidation equilibrium with a series of inactive metabolites (Pirog & Collins 1999). As a result, it is typically present in circulation in smaller quantities than other androgens (Luu-The & Laberie 2010; Penning et al 2000). This regulation may serve in part to limit adipogenic actions that have been chronically elevated DHT levels linked to metabolic and cardiovascular disease risk (Duskova & Pospisilova 2011). Given its heightened androgenic potency, the enzymatic machinery required for bioconversion of androgens to DHT is not universally distributed, allowing tissue specific effects only in selected androgen sensitive tissues (Thigpen et al 1993).

Emerging evidence suggests that exercise can acutely trigger local muscular conversion of testosterone to DHT in rats (Aizawa et al 2010). DHT has further been shown to enhance force production in isolated fast twitch muscle fibre bundles from mice via rapid non-genomic signalling (Hamdi & Mutungi 2011) and to promote a variety of transcriptional events associated with anabolism via classical genomic signalling (Yoshioka et al 2006). Historically, human skeletal muscle was not thought to contain significant levels of the 5 $\alpha$ -reductase responsible for conversion of testosterone to DHT (Thigpen et al 1993). However, this view has recently been challenged by the discovery of a third isoform of 5 $\alpha$ -reductase that is highly expressed in human skeletal muscle (Godoy et al 2011).

Given that the role of DHT in the acute adaptive response to exercise in humans is not known, it would be prudent to establish whether exercise provokes an increase in circulating DHT prior to any detailed exploration of its potential role in exercising muscle function. The purpose of the current study was therefore to determine whether circulating DHT is acutely elevated in a group of exercise trained men performing repeat sprint exercise that involves sustained recruitment of fast twitch muscle fibres. We further aimed to establish whether this exercise response was related to training status or characteristics of the sprint performance.

## **METHODS**

### **Participants**

Fourteen healthy men participated in this study (age  $28.3 \pm 4.1$  y; body mass (BM)  $75.6 \pm 8.5$  kg;  $VO_{2max}$   $61.0 \pm 8.1$  ml·kgBM<sup>-1</sup>·min<sup>-1</sup>). All participants completed a self-report training questionnaire and were categorized as either a recreational (n = 7) or a competitive (n = 7) athlete with a minimum training history of six years. These criteria were established in order to isolate the effects of training and competition status without the confounding effects of years of training history as both have been implicated in the modulation of the hormonal response to exercise (Crewther et al 2006). Sports participation included running, cycling, swimming, triathlon, combat sports, ball sports and team sports. Amongst the reported activities, all competitive athletes participated in cycling or triathlon and all but one of the recreational athletes were engaged in resistance training. The study received approval from the University of Bath Research Ethics Approval Committee for Health and each participant was



provided with a written and verbal briefing regarding the nature of the testing, and provided written, informed consent prior to participation.

### **Experimental Protocol**

*Incremental exercise trial:* In order to determine participant fitness and to set workloads for subsequent sprint exercise, all participants undertook an incremental exercise test to exhaustion on a stationary cycle ergometer (Schoberer Rad Messtechnik, SRM, Germany). Expired gases were collected in the final minute of exercise using a Douglas bag and subsequently analysed for volume of air expired using a dry gas meter (Harvard Apparatus, UK), temperature of expired gases via a digital thermometer (model C, Edale Instruments, UK) and fractional concentrations of O<sub>2</sub> and CO<sub>2</sub> using paramagnetic and infra-red methods, respectively (Servoflex MiniMP, Servomex, UK). These analysers were calibrated prior to each test with gases of known composition and volume within the physiological range, as certified by prior gravimetric analysis (British Oxygen Company, U.K), and measured values were used to determine the maximal rate of oxygen consumption (VO<sub>2max</sub>). Starting load was self-selected based on warm up intensity and the exercise protocol consisted of 30W increments every three minutes for fifteen minutes followed by 20W increments per minute until exhaustion. Power output during the final minute was averaged to represent work capacity (W<sub>max</sub>).

*Repeat sprint exercise trial:* On a subsequent visit, at least 48 hours later, participants completed a bout of sprint interval cycle exercise on the stationary ergometer (Schoberer Rad Messtechnik, SRM, Germany), consisting of 10 repetitions of 30 seconds sprinting at a target load of 150% of the  $W_{\max}$  determined from the incremental test, interspersed with 90 seconds of low intensity recovery cycling at or below 100W. In order to normalize for circadian variation in hormonal state (Hayes, Bickerstaff & Baker 2010) all sprint testing took place in the morning between 2 and 4 hours after waking. Workload was self-paced and participants were given real-time numerical and graphical feedback on their current power output, cycling cadence and time elapsed. Where participants were unable to sustain the target load they were encouraged to perform maximally during the remaining sprints.

*Blood sampling:* On arrival at the laboratory, a trained phlebotomist collected 5mL of venous blood from a superficial antecubital vein without stasis. Following this pre-exercise sample, further samples were collected 5 minutes post- and 60 minutes post-exercise. Samples were suspended in serum collection tubes (Serum Z/5 ml, Sarstedt, Germany) for 15 minutes before being centrifuged for 10 minutes at 1500g. Supernatant was immediately transferred to polypropylene Eppendorf tubes and frozen at  $-20^{\circ}\text{C}$  until analysis.

*Hormonal analysis:* Serum samples were analysed in duplicate for total testosterone, free testosterone, and DHT concentrations, using commercial ELISA kits (IBL,

Hamburg, Germany). All samples for a given participant were analysed on the same assay plate. Pooled intra-assay sample variance (CV) for duplicate samples was 4.5% for total testosterone, 5.6% for free testosterone and 5.3% for DHT and inter-assay sample variance for controls was 6.3% for total testosterone, 6.4% for free testosterone and 8.9% for DHT.

### **Statistical Analysis**

Demographic comparisons between athlete groups were conducted by one-way analysis of variance (ANOVA) and hormonal responses were analysed using a two way (group x time) repeated measures general linear model, with the Greenhouse-Geisser correction applied where assumptions of sphericity were violated. Main effects were isolated by pairwise multiple comparisons using the Bonferroni method. All hormonal data were normalized by natural log transformation prior to analysis. Hormonal changes from pre to post exercise for different androgens were then examined for correlation to each other and to demographic and sprint performance data by Pearson product moment correlation. All statistical analysis was conducted using IBM SPSS Statistics (Release 20.0.0, IBM, USA) and all data are presented as mean  $\pm$  SD. Statistical significance was set at the level of  $P < 0.05$ .

## RESULTS

### Participant fitness and training history

There were no significant differences in age or body mass between athlete groups categorized by competition status (Table 5.1). However, both  $VO_{2max}$  ( $P = 0.011$ ) and  $W_{max}$  ( $P = 0.002$ ) calculated during the incremental exercise protocol were significantly higher for competitive compared to recreational athletes. Competitive athletes also reported a significantly higher habitual training volume than recreational athletes (competitive v recreational:  $10.5 \pm 6.8$  hrs.week<sup>-1</sup> v  $3.7 \pm 0.9$  hrs.week<sup>-1</sup>;  $P = 0.022$ ) but with no difference in years of training history ( $10.3 \pm 5.2$  yrs v  $11.4 \pm 3.0$  yrs;  $P = 0.616$ ).

	Competitive Athlete	Recreational Athlete
Age (yrs)	28.5 ± 5.1	28.0 ± 3.1
Body Mass (kg)	74.3 ± 7.3	76.8 ± 10.0
$VO_{2max}$ (ml/min/kg)	66.1 ± 4.5	55.9 ± 7.8*
$W_{max}$	375.8 ± 44.5	297.7 ± 26.0**

Table 5.1. Participant characteristics (n=7 competitive, n=7 recreational), aerobic capacity ( $VO_{2max}$ ) and work capacity ( $W_{max}$ ). Competitive versus recreational athletes: \* =  $P < 0.05$ , \*\* =  $P < 0.01$ . Values are mean ± SD.

### Sprint performance

The competitive group completed the sprint task at the target load of 150% of measured work capacity (average power:  $149.4 \pm 10.1\%$   $W_{max}$ ). However, the

recreational group were less able to sustain the target load for all ten repetitions (average power:  $135.3 \pm 15.5\% W_{\max}$ ) although the difference between groups did not reach significance ( $P = 0.066$ ). As a result, competitive athletes performed at a significantly higher average power (competitive v recreational:  $560.1 \pm 62.1 W$  v  $404.1 \pm 68.4 W$ ;  $P = 0.001$ ) resulting in a greater work done during sprinting ( $168.0 \pm 18.6 kJ$  v  $121.2 \pm 20.5 kJ$ ;  $P = 0.001$ ) compared to recreational athletes.

### **Hormonal response to sprint exercise**

Significant elevations from pre- to 5' post-exercise occurred in the levels of total testosterone ( $P = 0.002$ ), free testosterone ( $P = 0.001$ ) and DHT ( $P = 0.022$ ), with all three parameters returning to pre-exercise levels by 60' post-exercise (Fig 5.1). Pre-exercise levels of free testosterone were correlated with levels of total testosterone ( $r = 0.689$ ;  $P = 0.006$ ) and DHT (Fig 5.2 Panel A,  $r = 0.536$ ;  $P < 0.05$ ). Changes in DHT levels with exercise (5' Post - Pre) correlated significantly with changes in levels of total testosterone ( $r = 0.870$ ;  $P < 0.001$ ) and free testosterone (Fig. 5.2 Panel B,  $r = 0.914$ ;  $P < 0.001$ ).

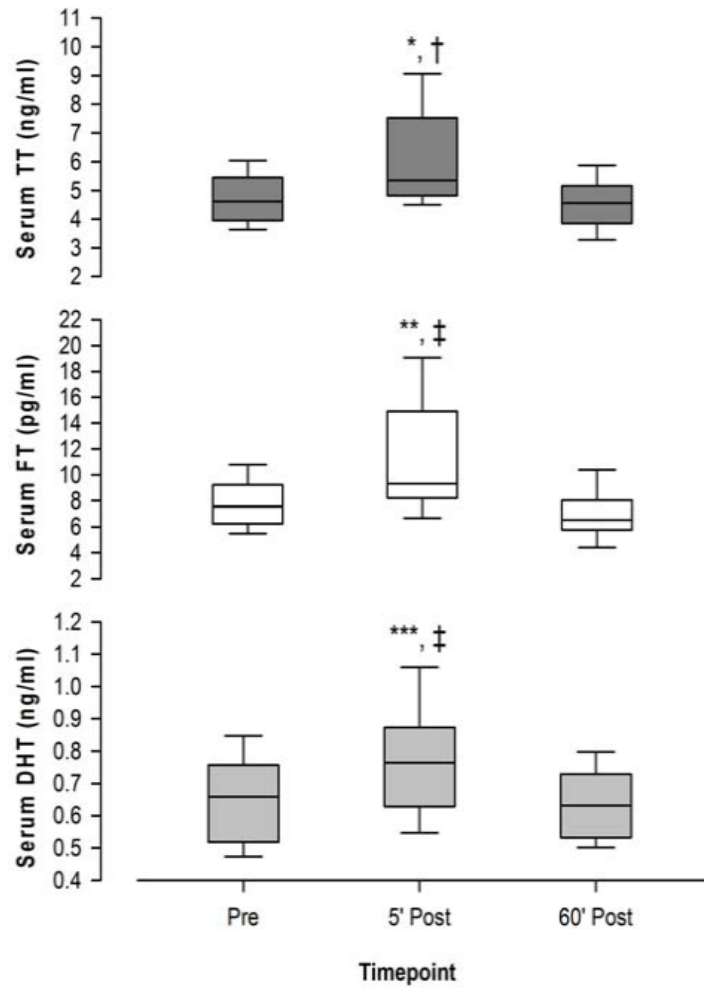


Figure 5.1. Serum TT (dark grey boxes), FT (white boxes) and DHT (light grey boxes) levels at three time points: pre-exercise, 5' post-exercise and 60' post-exercise. Box plots show median line, 25<sup>th</sup> - 75<sup>th</sup> percentile box and 10<sup>th</sup> - 90<sup>th</sup> percentile error bars. \* P = 0.002 v Pre, \*\* P = 0.001 v Pre, \*\*\* P = 0.022 v Pre, † P = 0.001 v 60' Post, †† P < 0.001 v 60' Post.

### Hormone response, training status and sprint performance

All hormonal responses were compared in relation to competitive status and no group effects or group x time interactions were found for competitive versus recreational athletes. However, the sprinting cadence averaged across all ten sprints

correlated with changes in FT ( $r = 0.574$ ,  $P = 0.032$ ) and the highest average sprinting cadence across an individual sprint bout was associated with changes in FT ( $r = 0.697$ ,  $P = 0.006$ ), DHT ( $r = 0.625$ ,  $P = 0.017$ ) and TT ( $r = 0.603$ ,  $P = 0.022$ ). Habitual weekly training volume correlated with the change in TT only ( $r = 0.569$ ,  $P = 0.034$ ). Hormonal exercise responses did not vary in relation to work capacity during sprinting or fitness status determined during the incremental exercise trial.

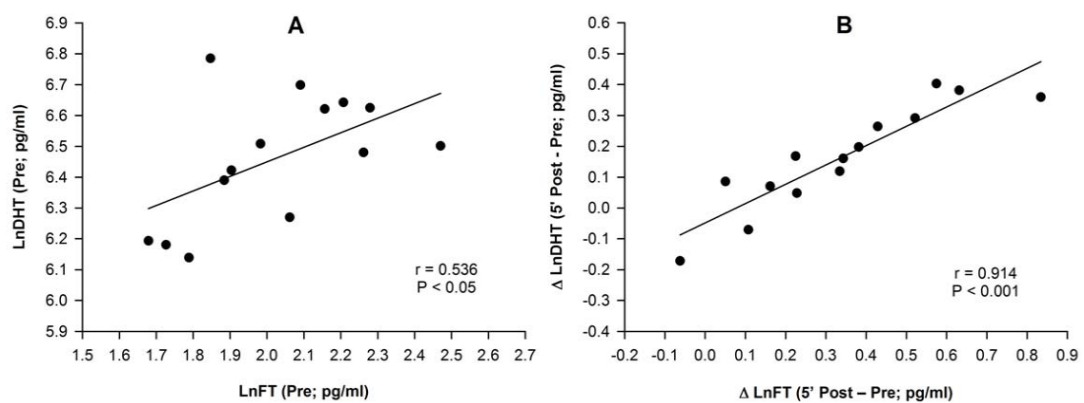


Figure 5.2. Correlations between log-transformed serum free testosterone and serum DHT: A - pre-exercise log-levels ( $r = 0.536$ ,  $P < 0.05$ ); B - change in log-levels with exercise (5' post – pre;  $r = 0.914$ ,  $P < 0.001$ ).

## DISCUSSION

To our knowledge this is the first report of the presence of increased circulating levels of the androgen DHT during the period immediately following an exercise stimulus in humans. Elevations in DHT were short lived, returning to baseline levels within one hour after exercise. This timescale of action is consistent with previous reports for testosterone (Deschenes et al 1991), whilst adding DHT to the array of androgenic factors elevated in the acute phase response to exercise. The presence of elevated



DHT during exercise may be of considerable significance given that DHT has a greater affinity than testosterone at the androgenic receptor and creates a more stable androgen-receptor complex (Bauer et al 2000, Grino, Griffin & Wilson 1990). That these elevations were short lived may be of equal importance in determining their functional relevance.

The short acting effects of androgens during exercise have attracted recent attention (Crewther et al 2011). Elevations in testosterone during exercise are thought to play a role in supporting the maximal expression of powerful muscular force production, linking motivational behaviour with functional output (Cook & Crewther 2012), but this relationship appears to depend upon training status (Crewther et al 2012a). Whether these modulatory effects of training extend to the activation of 5 $\alpha$ -reductase in exercising skeletal muscle is not yet known. Modulatory increases in skeletal muscle DHT levels in response to exercise training have recently been shown in rodents (Aizawa et al 2011) and chronic up-regulation of basal circulating DHT levels with training has also been reported in humans (Hawkins et al 2008). However, acute exercise responses were not reported. In the present study a significant increase in circulating DHT was observed following sprint exercise in a group of healthy young men with a history of six or more years of exercise training. Neither performance capacity in the tests administered nor the habitual training volume of these athletes discriminated the degree of DHT response. Whilst these findings are in conflict with previous reports indicating that training exposure can modulate the androgen response to exercise (Crewther et al 2006), this may be unsurprising given

the mixed athletic background of this cohort. A similar divergence has been shown in other mixed ability athletic groups, suggesting that androgen responsiveness may only be pertinent to functional output in a subgroup of athletes (Crewther et al 2012a; Crewther et al 2012b). Future studies should examine acute DHT responses to exercise in less well trained or sedentary individuals as well as exploring the modulation of these responses and their relationship to functional gain with the imposition of an exercise training program.

In the trained young men studied here we found an association between the highest volitional cadence maintained for thirty seconds during sprint cycling and the degree of responsiveness to the sprint stimulus of all three of the measured androgens, including DHT. Whilst lacking causation, this correlation points towards the possibility that phenotypical characteristics of athletic ability may play a role in determining the hormonal response to sprinting. In trained cyclists, the proportion of fast twitch fibres by cross-sectional area of vastus lateralis muscle, an aspect of athletic phenotype known to carry a significant degree of heritability (Simoneau & Bouchard 1995), correlates strongly with optimal sprinting cadence and to a lesser degree with maximal sprinting power output (Hautier et al 1996). This relationship holds with the age related decline of muscle function in older men (Pearson et al 2006). At the cellular level DHT has been shown to increase transcriptional signalling of factors involved in ATP production and calcium cycling in mouse skeletal muscle (Yoshioka et al 2006). In the rat, cultured skeletal muscle cells exposed to testosterone or dehydroepiandrosterone (DHEA) exhibit a DHT conversion

dependent enhancement of glucose metabolism by increasing protein expression and translocation of GLUT-4 (Sato et al 2008) and administration of DHT to isolated muscle fibre bundles enhances contractile function, but only in fast twitch fibres (Hamdi & Mutungi 2010). It is possible therefore that the androgenic response to sprint exercise in men may depend to some degree on the morphology of active muscle. Alternatively, sprint cadence and androgen status may be covariates of an underlying driver related to other characteristics of the exercise response, such as changes in contractile function and cellular metabolism resulting from alterations in anisotonic compartmental fluid dynamics (Cermak et al 2009).

The tight coupling of exercise induced increases in total and free testosterone and DHT in the present study (Fig 5.2) is suggestive of a common pathway of androgenic promotion during repeat sprint exercise, with skeletal muscle a likely candidate in driving this response. In support of this view, preliminary findings from our group indicate that elevations in DHT can also be detected in human muscle dialysate during high intensity exercise (unpublished data). In rodents exercise has been shown to promote active bioconversion of androgens by skeletal muscle resulting in temporary elevations in both DHT and testosterone of a similar magnitude and timeframe to those seen here (Aizawa et al 2010). However, it is currently not known whether human skeletal muscle is capable of local androgenic bioconversion in the face of an exercise stimulus. Until recently, it was widely thought that human skeletal muscle lacked expression of the 5 $\alpha$ -reductase responsible for conversion of testosterone to DHT (Stuerenburg & Schoser 1999; Thigpen et al 1993). Empirical

data directly supporting these claims in normal, healthy humans is scarce in the available literature, with inferences regarding 5 $\alpha$ -reductase inactivity largely dependent upon observations of normal muscular development in patients with congenital 5 $\alpha$ -reductase deficiency (Gormley 1995; Rittmaster 1994), and evidence of sustained muscle hypertrophy during exogenous testosterone administration in the presence of 5 $\alpha$ -reductase inhibition in testosterone deficient men (Page et al 2005). Crucially, the provenance of tissue samples from the available studies directly examining 5 $\alpha$ -reductase in healthy humans is unclear, leaving open the prospect that expression of 5 $\alpha$ -reductase is actively modulated in skeletal muscle by factors such as phenotype, age and exercise training. This view is strengthened by recent reports of a third isoenzyme of 5 $\alpha$ -reductase that shows an expression profile in human skeletal muscle (Godoy et al 2011, Yamana, Labrie & Luu-The 2010). Further studies are required to explicitly address this question in humans by conducting muscle biopsies following an exercise stimulus to determine both the changes in local androgen metabolism and the fibre type specificity of this response.

Much debate surrounds the functional role of androgens in the anabolic response to exercise training (Phillips 2012, Rønnestad, Nygaard & Raastad 2012). Whilst beyond the scope of this study, future investigations should consider the potential relevance of DHT to exercise induced anabolism, since administration of DHT to isolated intact muscle fibre bundles from the rat promotes protein accretion (Hamdi & Mutungi 2011), with effects mediated by mitogen-activated protein kinase (MAPK), and activates a transcriptome signature highly supportive of anabolic pathways in an

equivalent mouse model (Yoshioka et al 2006). If DHT is found to be of relevance to anabolic signalling then consideration should also be given to the resilience of the exercising DHT response under chronic training load. Depletion of circulating androgen levels is a feature of the continued high level stimulation of an intense conditioning exercise program (Hakkinen & Pakarinen 1991). Since 5 $\alpha$ -reduction of testosterone to DHT is an irreversible step in androgen metabolism, necessitating the inactivation of DHT following a stimulus (Penning et al 2000), the repeated elevation of DHT in response to the day-to-day demands of an intense conditioning exercise program may place a strain on androgen metabolism with potential implications for maintenance of future training load and subsequent adaptive response. That the habitual training volume of participants in the current study was related to the TT response to repeat sprint exercise could be indicative of an adaptation designed to sustain circulating androgen levels under training load.

This study represents a small staging post in the examination of the role of DHT in exercising muscle function. Extension of the findings presented here is limited by the small cohort studied and the extensive training history of participants. Whether the relationships reported would hold for more diverse groups encompassing sedentary or less well trained individuals and highly trained elite athletes, or for different modes or durations of exercise, was beyond the scope of the current study. A further note of caution is required in comparing the acute responses of this group since there may have been variable adherence to advice regarding the control of training load in the days before the sprint trials took place. It remains possible that residual training

fatigue differentially influenced both the ability of participants to perform optimally and the extent of their hormonal response.

## **Conclusion**

Repeat sprint exercise is capable of inducing an increase in circulating DHT in healthy active young men, particularly when sprinting cadence is high. It would be of significant value to determine whether DHT is converted locally from other androgens by active muscle during exercise and what factors might determine the extent of the exercising DHT response, such as age, sex, competitive athletic status, exercise training history and characteristics of the exercise stimulus. The role of DHT in particular, and the HPA axis in general, in modulating muscle function during exercise training and sports performance is worthy of further investigation.

## Chapter 6: The Effects of Ketone Ester Consumption on Repeat Sprint Performance

### Following Pre-Fatigue in Well-Trained Cyclists

#### ABSTRACT

Sustaining repeated sprint performance under fatigue is an important requirement for many sports and in particular for a number of outdoor endurance sports. Whilst ketone ester consumption has been shown to improve endurance performance during time trial style exercise (Cox et al.,2016) it is not currently known whether nutritional ketosis can benefit more intermittent endurance performance. This study aimed to determine the impact of ketone ester consumption, at the upper end of the recommended dosing range (10mg/min/kg-body-mass) on repeat sprint cycling performance following pre-fatigue. Fourteen well trained men performed three randomised trials of 20-min steady state (SS) cycling at 80% of 20-min work capacity followed immediately by ten 30s sprints with 90s recovery. Trials were performed following consumption of water (H<sub>2</sub>O), a ketone ester drink (KET) or a calorie matched carbohydrate drink (CHO). Compared to CHO, KET resulted in a 2.1% decrement in average sprint power ( $P<0.01$ ) and attenuated exercise induced increases in plasma lactate ( $P<0.001$ ), glucose ( $P<0.001$ ), NEFA ( $P<0.001$ ), dihydrotestosterone (DHT;  $P=0.07$ ) and testosterone/cortisol ratio (T/C;  $P=0.07$ ), whilst elevating plasma cortisol ( $P<0.05$ ). The difference between KET and CHO in the T/C ratio response to exercise correlated with the difference in average sprint power ( $P<0.05$ ). In conclusion, nutritional ketosis diminished repeat sprint performance with significant effects on glycolytic and hormonal state.



## INTRODUCTION

A ketone ester drink has recently been developed that can raise ketone body concentration in the body, when consumed as part of a normal diet, to those normally only seen following several days of fasting (Clarke et al., 2012). The metabolic milieu following ketone ester consumption represents a novel nutritional state given that ketosis is achieved in the absence of calorie deficit or carbohydrate restriction, and their ensuing complications (Kwiterovich, Jr et al., 2003). As a result, increasing ketone body availability during eucaloric exercise has the potential to profoundly alter exercise metabolism (Cox, 2013; Cox et al., 2016) and initial laboratory performance trials indicate that nutritional ketosis may confer ergogenic benefit to sustained endurance performance of 30-minutes duration or more (Cox, 2013) and to mixed intensity gameplay simulation of 90-minutes duration (unpublished data).

Early work in this research programme has indicated that there may be a lower threshold of ketone ester dosing required for efficacy and a need for exercise to be of sufficiently high intensity to elicit a significant degree of metabolic stress (Chapter 4). Whilst the exercise protocol adopted in chapter 4 was intermittent in nature, it likely failed to fully represent the workload excursions more typical of outdoor endurance sports (Sharma et al., 2015) where large fluctuations in workload are interspersed with sustained sprint style efforts. The degree of ecological validity conferred by such workload selection is a critical factor for translation of laboratory data into the field (Atkinson & Nevill, 2001).

Work conducted with the ketone ester to date has largely involved participants performing in isolation in concurrence with the majority of research exploring novel nutritional interventions. However, such approaches confer a motivational climate that is far removed from the demands of real world sports performance. Viewed from the perspective of achievement goal theory, elite athletes are motivated by both internal mastery and external performance (Pensgaard & Roberts, 2000). Further, achievement goal orientation has been shown to influence the assessment of aerobic capacity in a manner dependent upon the orientation of the motivational climate in the laboratory (Buch et al., 2016), suggesting a critical role not just for motivation but for climactic attendance to motivation in performance assessment. This view is further supported by evidence for a positive impact of head to head competition upon cycling time trial performance when compared to performing in isolation, even when the competition was virtual rather than real (Corbett, Barwood, Ouzounoglou, Thelwell, & Dicks, 2012b).

This study aims to determine whether ketone ester feeding can improve the ability to sustain repeated sprinting under fatigue, a key performance requirement for a number of outdoor endurance sports. Ketone ester will be fed at the upper end of the recommended dosing range (10mg/kg body mass/min of exercise) to address previous concerns regarding a minimal threshold dose for efficacy. In order to go some way to addressing the issue of performance motivation in laboratory trials, the current study will place participants in a group dynamic where they will perform alongside their peers whilst also being incentivised with external rewards for both individual and group performance.

## **METHODS**

### **Participants**

Fourteen healthy men participated in this study (age  $31.9 \pm 6.8$  yrs; height  $179.0 \pm 7.6$  cm; body mass (BM)  $77.5 \pm 9.3$  kg). All participants completed a self-report training questionnaire and were categorised as 'recreational' or 'competitive' cyclists or triathletes. The study received approval from the University of Bath Research Ethics Approval Committee for Health and each participant was provided with a written and verbal briefing regarding the nature of the testing, and provided written, informed consent prior to participation.

### **Experimental Design**

This study followed a double-blinded, randomised cross-over design. Participants attended the laboratory on six occasions each separated by 7 days. In order to normalize for circadian variation in hormonal state (Hayes, Bickerstaff, & Baker, 2010) all testing took place at the same time of the evening each week. Diet and exercise were recorded for the 24 hours before the first trial and participants were asked to repeat this routine before all subsequent trials. All exercise was conducted on air-braked, stationary cycle ergometers (Wattbike Pro, UK) under controlled laboratory conditions at 20°C and 50% humidity. Wattbike manufacturer reported accuracy is better than  $\pm 1.0\%$  at the power outputs reported here and reliability trials indicate a coefficient of variation of 2.4% for mean power on a single 30-second sprint (see General Methods). Participants were free to use their own cycling pedals

and shoes with the stipulation that the same equipment be used across all trials. Cycling geometry (vertical and horizontal positioning of both handle bars and saddle) was recorded for each participant on the first visit and replicated on all subsequent trials.

## **Experimental Protocol**

### *Preliminary Trial*

On the first preliminary trial day all participants performed a 20-minute maximal cycling time trial followed, after a period of recovery, by 10 sprint efforts of 30-seconds duration, with the aim of determining participant work capacity whilst also achieving familiarisation with the test procedure for subsequent trials. For the 20-minute TT workload was self-paced and participants were given real-time numerical feedback on their power output, cycling cadence and time elapsed. Participants were encouraged to complete as much work as possible across the 20-minutes. For the subsequent sprints participants were encouraged to work close to maximum effort in order to become familiar with the pacing of the sprints and the mechanics of the bicycle ergometer.

### *Familiarisation Trials*

On two subsequent familiarisation days participants performed 20-minutes of steady-state cycling at 80% of their work capacity from the preliminary trial, designed

to elicit some pre-fatigue (Thomas, Elmeua, Howatson, & Goodall, 2016), followed by 10 sprint efforts of 30-seconds duration with 90-second rest periods between. Sprint workload was self-paced and participants were given real-time numerical feedback on their power output, cycling cadence and time elapsed. Participants were encouraged to complete as much work as possible across the ten sprints.

### *Main Trials*

For the three main trials participants followed the same trial procedure as the familiarisation days whilst also being randomly assigned drinks containing either 6.1mg/min/kg-body-mass carbohydrate + 10mg/min/kg-body-mass ketone ester (KET), 6.1mg/min/kg-body-mass + further carbohydrate at the calorific equivalent of the ketone ester arm (CHO) or an equivalent volume of water (H<sub>2</sub>O). Carbohydrate was provided in accordance with current sports nutrition guidelines in the form of glucose and fructose in the ratio 2:1 (Currell & Jeukendrup, 2008). Drinks were blinded by colour and flavour matching and were also matched for volume by the addition of water. All experimental drinks were consumed in a single bolus 45-minutes before the onset of exercise and further water was provided ad-libitum during all trials.

### *Group Dynamics*

On each visit participants exercised in groups of 6-8, making two groups who visited the laboratory on separate days. Ergometers were arranged in a circle so that

participants could see each other as they performed. In addition, both individual and group incentives were provided in the form of financial credits for sports nutrition products. Participants received rewards based on their average power output over the ten sprints relative to their best score from the familiarisation trials. Rewards were provided each week for the best individual and group relative sprint score and an enhanced reward was provided at the end of the study for the best individual and group score over all three trials. Participants were therefore incentivised to deliver maximal performances on each trial visit for the benefit of both themselves and their immediate peers in order to create a motivational climate for performance.

#### *Blood Sampling and Analysis*

On arrival of each participant at the laboratory, a trained phlebotomist inserted a venous cannula into an antecubital vein without stasis. Blood samples (~5ml) were drawn and inserted into EDTA collection tubes (EDTA 5ml, Sarstedt, Germany) at baseline, prior to the onset of exercise, immediately after the 20-minute bout of cycling and following the 4<sup>th</sup> and 10<sup>th</sup> sprint efforts. Blood was then centrifuged and the plasma fraction retained (thereby rendering the sample acellular). Plasma samples were immediately transferred to polypropylene Eppendorf tubes and frozen at -80°C for later analysis of glucose, lactate, glycerol, free fatty acids, ketone bodies and steroid hormones. Plasma metabolites (lactate, glucose, non-esterified fatty acids (NEFA), glycerol and betahydroxybutyrate (BHB)) were analysed by spectrophotometry using commercially available analysis kits (RX Daytona, Randox, Crumlin, UK) and steroid hormones (total testosterone (TT), dihydrotestosterone

(DHT) and cortisol (C)) were analysed in duplicate using commercial ELISA kits (TT+C: IBL, Hamburg, Germany; DHT: MyBioSource, USA).

### *Subjective Ratings*

During all trials participants were asked to provide regular subjective ratings of their perceived exertion levels, affective state and gastrointestinal comfort (see General Methods), whilst perceptions of drink pleasantness were recorded immediately following ingestion of each trial drink.

### **Statistical Analysis**

A paired two-tailed t test was used to identify differences between treatments, and a two-way (treatment x time) general liner model for repeated measures was employed for all serial measures, with the Greenhouse-Geisser correction applied where assumptions of sphericity were violated. Main effects were isolated by pairwise multiple comparisons using the Bonferroni method. Blood metabolites and hormonal responses were then examined for correlation to each other and to sprint performance data by Pearson product moment correlation. All statistical analysis was conducted using IBM SPSS Statistics (Release 20.0.0, IBM, USA) and all data are presented as mean  $\pm$  SD. Statistical significance was set at the level of  $P < 0.05$ .



## RESULTS

### Participant fitness and training history

Participant fitness was demonstrated by a group average work capacity on the 20-minute time trial of  $271.0 \pm 48.4\text{W}$  and a body weight corrected work capacity of  $3.5 \pm 0.7\text{W/kg}$ . All participants were self-rated as either a recreational athlete or a top-level/competitive athlete with seven participants holding a British Cycling racing license. Training history data indicated that the group on average had been in regular exercise training for  $11.9 \pm 7.1$  years, had been in regular cycling training for  $4.4 \pm 2.5$  years and currently completed an average weekly cycle training volume of  $9.3 \pm 6.0$  hours.

### Sprint Performance

During familiarisation trials the best average sprint power for the group was  $516.6 \pm 73.8\text{W}$  with no significant difference between the first and second familiarisation visits. Average sprint power during the CHO ( $518.1 \pm 72.9\text{W}$ ) and H<sub>2</sub>O ( $515.8 \pm 70.2\text{W}$ ) trials was not significantly different from the best familiarisation trial, however, during the KET trial sprint power ( $507.3 \pm 73.3\text{W}$ ) was significantly lower than both the best familiarisation trial ( $P < 0.05$ ) and the CHO trial (Fig 6.1:  $P < 0.01$ ).

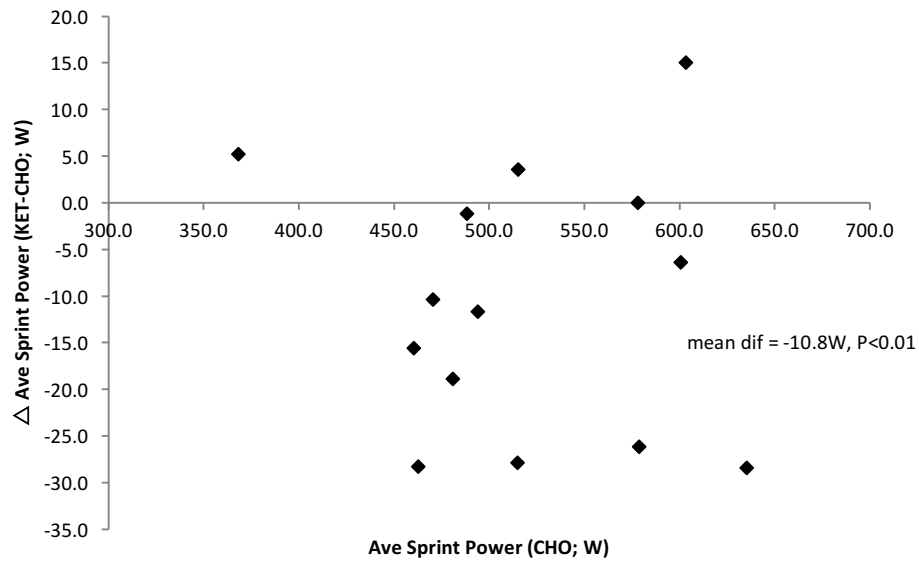


Figure 6.1. Difference between trials (KET-CHO) in average sprint power versus average sprint power in the CHO trial (n=14 male cyclists). Mean difference = -10.8W, P<0.01.

### Metabolic and Hormonal Responses

Blood plasma metabolite and hormonal responses are shown in Table 6.1. On all trial limbs there were significant increases in plasma lactate (H2O: P<0.001; KET: P<0.001; CHO: P<0.001), glycerol (H2O: P<0.001; KET: P<0.001; CHO: P<0.001) and testosterone (H2O: P<0.01; KET: P<0.01; CHO: P<0.01) following completion of ten sprints. In addition, during the water and carbohydrate trials plasma glucose (H2O: P<0.01; CHO: P<0.01) and DHT (H2O: P<0.01; CHO: P<0.05) were increased post sprints and during the ketone ester (KET) trial plasma NEFA was decreased (P<0.01) and both BHB (P<0.001) and cortisol (P<0.01) were increased post sprints.

	Water (H <sub>2</sub> O)		Ketone Ester (KET)		Carbohydrate (CHO)	
	baseline	post sprints	baseline	post sprints	baseline	post sprints
Lactate (mmol/l)	1.3 ± 0.4	16.4 ± 3.0 <sup>***</sup>	1.3 ± 0.6	15.1 ± 2.1 <sup>***,†</sup>	1.2 ± 0.4	17.0 ± 2.1 <sup>***,‡‡‡</sup>
Glucose (mmol/l)	5.0 ± 0.7	6.3 ± 1.4 <sup>**</sup>	4.8 ± 0.7	5.4 ± 1.3 <sup>†††</sup>	4.9 ± 0.5	6.1 ± 1.4 <sup>**‡‡‡</sup>
NEFA (mmol/l)	0.17 ± 0.10	0.27 ± 0.20 <sup>l</sup>	0.13 ± 0.09	0.07 ± 0.04 <sup>*,††</sup>	0.15 ± 0.12	0.11 ± 0.08 <sup>‡‡‡</sup>
Glycerol (umol/l)	32.1 ± 15.9	160.3 ± 52.3 <sup>***</sup>	27.9 ± 10.3	136.9 ± 60.2 <sup>***</sup>	31.9 ± 11.0	152.7 ± 53.3 <sup>***</sup>
BHB (mmol/l)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	2.3 ± 0.8 <sup>***,†††</sup>	0.1 ± 0.0	0.1 ± 0.0 <sup>‡‡‡</sup>
Testosterone (ng/ml)	2.79 ± 0.91	3.52 ± 1.16 <sup>**</sup>	2.69 ± 0.92	3.53 ± 1.10 <sup>**</sup>	2.59 ± 0.86	3.46 ± 0.93 <sup>**</sup>
DHT (pg/ml)	991.5 ± 444.8	1138.3 ± 521.4 <sup>**</sup>	1005.6 ± 496.6	1080.6 ± 585.5	1096.1 ± 520.0	1175.6 ± 574.8 <sup>*,†</sup>
Cortisol (ng/ml)	57.7 ± 14.5	63.7 ± 23.3	63.9 ± 20.1	97.4 ± 40.7 <sup>*,††</sup>	60.8 ± 17.1	74.2 ± 37.9 <sup>†</sup>
Testosterone/Cortisol	0.050 ± 0.018	0.061 ± 0.029	0.047 ± 0.026	0.048 ± 0.040 <sup>†</sup>	0.046 ± 0.018	0.060 ± 0.044 <sup>†</sup>

Table 6.1. Blood plasma metabolite and hormonal responses at baseline and after all ten sprints for water (H<sub>2</sub>O), ketone ester (KET) and carbohydrate (CHO) trials (n=14 male cyclists). Post sprints v baseline: \* = P<0.05, \*\* = P<0.01, \*\*\* = P<0.001; H<sub>2</sub>O v CHO: <sup>l</sup> = P<0.05; H<sub>2</sub>O v KET: <sup>†</sup> = P<0.05, <sup>††</sup> = P<0.01, <sup>†††</sup> = P<0.001; KET v CHO: <sup>+</sup> = P=0.07, <sup>‡</sup> = P<0.05, <sup>‡‡‡</sup> = P<0.001.

Post sprints plasma NEFA was higher after the water trial than the carbohydrate trial with no further differences between those trials. Compared to the water trial, in the ketone ester trial post sprints plasma lactate (P<0.05), glucose (P<0.001), NEFA (P<0.01) and testosterone/cortisol ratio (P<0.05) were all decreased and both BHB (P<0.001) and cortisol (P<0.01) were increased. Compared to the carbohydrate trial, in the ketone ester trial post sprints plasma lactate (P<0.001), glucose (P<0.001), NEFA (P<0.001), dihydrotestosterone (P=0.07) and testosterone/cortisol ratio (P=0.07) were all decreased and both BHB (P<0.001) and cortisol (P<0.05) were increased.

### Subjective Ratings

Subjective ratings of perceived exertion (RPE) and affect are summarised in Table 6.2. Perceived affect declined significantly from baseline in all trials with a trend for a greater fall in the ketone ester trial that did not reach significance. RPE rose

significantly in all trials from ‘fairly hard’ levels after steady state cycling to near maximal values after ten sprints (H2O:  $P < 0.001$ ; KET:  $P < 0.001$ ; CHO:  $P < 0.001$ ). There was a small but significant difference between the water and carbohydrate trials for RPE after sprint ten ( $P < 0.05$ ) but no further differences in Affect or RPE between trials.

		Water (H2O)	Ketone Ester (KET)	Carbohydrate (CHO)
	Baseline	2.7 ± 1.4	2.6 ± 1.6	2.7 ± 1.5
	PreSS	2.8 ± 1.4	2.3 ± 1.6	2.6 ± 1.5
Affect	PostSS	1.3 ± 1.6**	0.9 ± 1.5**	1.4 ± 1.7*
	PostSp4	0.1 ± 2.0***	-1.2 ± 1.6***	-0.4 ± 1.7***
	PostSp10	-2.3 ± 1.8***	-2.9 ± 1.5***	-2.4 ± 1.8***
	PostSS	13.1 ± 2.3	12.9 ± 1.9	12.7 ± 2.0
RPE	PostSp4	15.6 ± 1.7***	16.6 ± 1.6***	16.2 ± 1.6***
	PostSp10	18.5 ± 1.5*** <sup>l</sup>	18.9 ± 1.0***	19.1 ± 1.1***

Table 6.2. Subjective ratings of Affect and RPE at baseline, before steady state cycling (PreSS), after steady state cycling (PostSS), after four sprints (PostSp4) and after ten sprints (PostSp10) for water (H2O), ketone ester (KET) and carbohydrate (CHO) trials (n=14 male cyclists). Affect timepoint v baseline/RPE timepoint v PostSS: \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ ; H2O v CHO: <sup>l</sup> =  $P < 0.05$ .

The sum of self-reported gastrointestinal symptoms across the exercise period was significantly elevated from baseline in all trials as shown in Table 6.3. However, the GI symptom sum during exercise was significantly higher in the ketone ester trial than the water ( $P < 0.05$ ) and carbohydrate ( $P < 0.05$ ) trials. In particular, upper GI symptom sum during exercise was significantly higher in the ketone ester trial than the water ( $P < 0.01$ ) and carbohydrate ( $P < 0.05$ ) trials.

		Water (H <sub>2</sub> O)	Ketone Ester (KET)	Carbohydrate (CHO)
GI Sum	Baseline	0.3 ± 0.8	0.2 ± 0.4	0.2 ± 0.6
	Exercise	3.0 ± 3.5**	8.3 ± 8.5**, <sup>†</sup>	3.6 ± 4.8* <sup>‡</sup>
Upper GI Sum	Exercise	0.5 ± 1.3	4.9 ± 5.2 <sup>††</sup>	1.5 ± 2.2 <sup>‡</sup>
Lower GI Sum	Exercise	0.1 ± 0.3	1.1 ± 2.7	0.2 ± 0.6
Systemic GI Sum	Exercise	2.4 ± 3.5	2.3 ± 5.1	1.9 ± 3.4

Table 6.3. Summation of gastrointestinal symptom reports (GI Sum) split by upper (Upper GI Sum), lower (Lower GI Sum) and systemic (Systemic GI Sum) symptoms at baseline and across the exercise period for water (H<sub>2</sub>O), ketone ester (KET) and carbohydrate (CHO) trials (n=14 male cyclists). Exercise v baseline: \* = P<0.05, \*\* = P<0.01; H<sub>2</sub>O v KET: <sup>†</sup> = P<0.05, <sup>††</sup> = P<0.01; KET v CHO: <sup>‡</sup> = P<0.05.

Water was well tolerated by all participants but both the ketone ester and the carbohydrate drink were rated as ‘strongly unpleasant’ to ‘very strongly unpleasant’ with no significant difference between the two. When asked upon exit interview to identify which was the ketone ester drink, 60% of participants guessed correctly.

### Sprint Performance Correlates

The difference in average sprint power between the carbohydrate and ketone ester trials correlated significantly with the change in T/C ratio from baseline to post sprint ten (Fig 6.2B: r = 0.538; P = 0.047) and trended towards correlation, without reaching significance, with cortisol levels after sprint ten (r = -0.513; P = 0.061), the T/C ratio after sprint ten (Fig 6.2A: r = 0.472; P = 0.088) and the change in testosterone levels from baseline to post sprint ten (r = 0.468; P = 0.092). The T/C ratio after sprint ten correlated significantly with self-reported affect after sprint ten (Fig 6.2C: r = 0.743; P = 0.002) and with GI symptom sum after sprint ten (Fig 6.2D: r = -0.836; P = 0.0002).

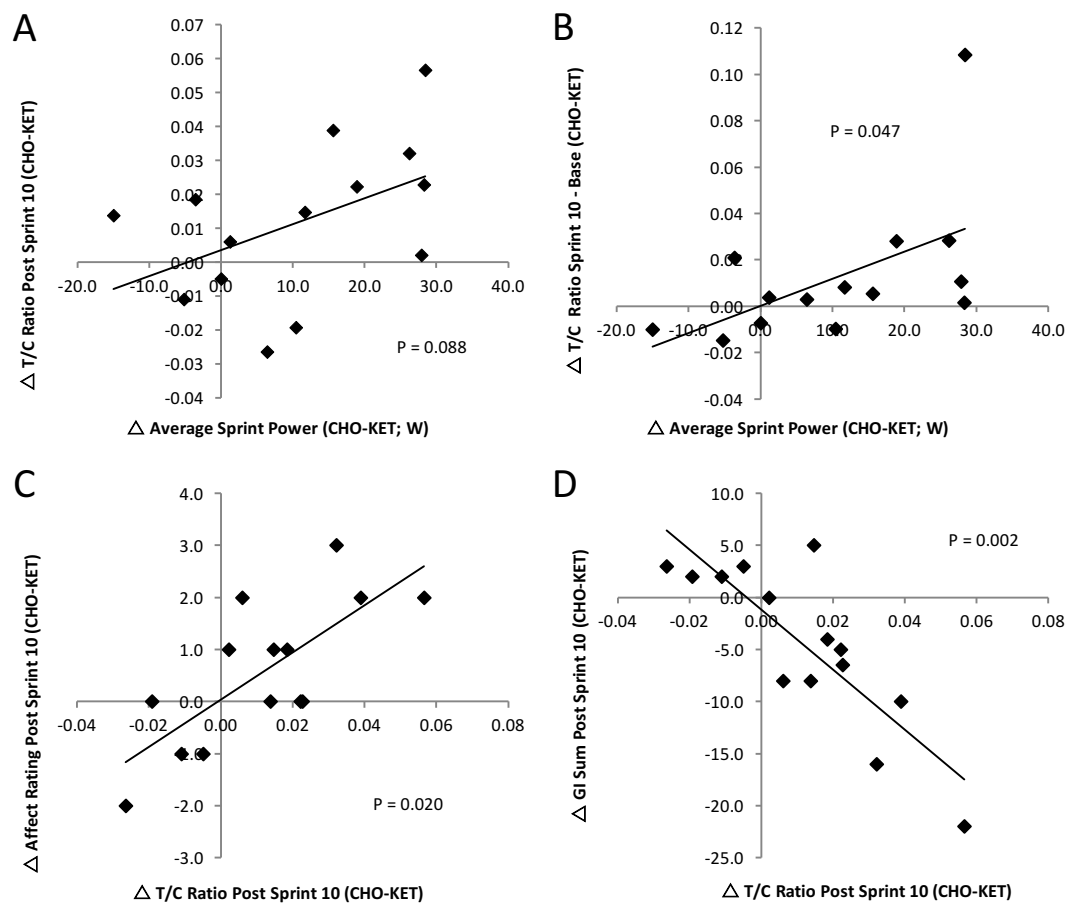


Figure 6.2. Correlation plots (n=14 male cyclists): A. difference in average sprint power between CHO and KET trials v difference in T/C ratio after sprint ten (P = 0.088); B. difference in average sprint power between CHO and KET trials v difference in T/C ratio change from baseline to sprint ten (P = 0.047); C. difference in T/C ratio after sprint ten between CHO and KET trials v difference in affect after sprint ten (P = 0.02); D. difference in T/C ratio after sprint ten between CHO and KET trials v difference in GI symptom sum after sprint ten (P = 0.002).

## DISCUSSION

The consumption of a ketone ester drink prior to exercise performance in this study resulted in a reduction in repeat sprint cycling performance. Whilst the genesis of this performance impairment remains complex, this finding should sound a general

note of caution regarding the utility of nutritional ketosis for sport performances requiring sustained sprint effort with limited recovery between sprints. This result sits in contrast to recent findings regarding the potential ergogenic utility of nutritional ketosis for longer duration exercise performance (Cox et al., 2016) and points to a potential threshold duration and/or intensity of effort, that has yet to be fully determined, through which ketosis may reverse its effects on performance. Unlike many other nutritional interventions for sports performance, where evidence for benefit may be equivocal but there is minimal evidence for harm (Jeukendrup, 2004), the findings presented here of a significantly detrimental performance effect suggest that particular care should be taken when considering ketogenic interventions in the sports arena.

Compared to the consumption of either water or a carbohydrate drink, consuming a ketone ester drink prior to exercise induced a moderate and sustained ketosis that persisted throughout the exercise period. In support of previous work with the same ketone ester (Cox et al., 2016) this ketotic milieu resulted in the suppression of exercise induced elevations in circulating lactate, glucose and free fatty acids (NEFA). Such changes would indicate that the presence of significant levels of circulating ketone bodies during exercise altered either or both of the mobilisation and oxidative fate of both glycolytic and lipolytic substrate. Whilst fuel selection appears a likely candidate for underpinning the ergogenic potential of nutritional ketosis during longer duration exercise (Cox & Clarke, 2014, Cox et al., 2016), in the present study the suppression of peak exercise plasma lactate levels with ketosis may be of significant import in explaining the decrement seen in power output during repeated

sprint effort. Sustained sprint activity has long been known to be reliant upon peak rates of glycolysis (Jacobs, Tesch, Karlsson, & Dotan, 1983), suggesting that anything that might inhibit glycolytic flux may have the potential to impair sprint performance. The degree of suppression in peak lactate levels seen here in the presence of ketosis did not correlate significantly with differences in average sprint power between trials. However, the hypothesis that nutritional ketosis may act as an inhibitor of peak glycolytic flux during sprint exercise is an attractive one that is deserving of further investigation.

Nutritional ketosis also impacted adversely on HPA axis signalling in the current study with an elevation in cortisol levels and a dampening of both dihydrotestosterone and the testosterone/cortisol ratio at peak exercise compared to carbohydrate feeding. Compared between trials, the change in T/C ratio with exercise correlated significantly with the change in sprint performance indicating that the shifting dynamics of HPA axis activity during exercise were predictive of performance outcome. The HPA axis is a ubiquitous signalling system with an array of modulating inputs and wide ranging output effects during exercise (Crewther et al., 2011), which likely include a degree of modulatory influence over maximal sprinting in athletes and an emerging role for dihydrotestosterone in particular as a potentiator of sprint performance (A. a Smith et al., 2013). Of note, ketosis induced differences in the T/C ratio at peak exercise were also correlated with differences in peak exercise affect and gastro-intestinal symptom reporting, indicating that HPA axis signalling was also significantly related to perceptions of well-being and GI tolerance of the ketone ester drink. Ketone ester feeding resulted in significantly greater GI symptoms, with



increases in upper GI symptoms in particular, and a non-significant trend towards worsening affect during exercise that may have contributed to performance impairment, perhaps in part through the influence of HPA axis signalling (Cook & Crewther, 2012a).

The participants in this study were all regular cyclists with a prolonged history of moderate level cycle training and varying levels of engagement in competitive cycling. However, it is clear from their training history and performance capacity that these were not highly trained, elite athletes. It is tempting to speculate that the detrimental effects of nutritional ketosis on sprint performance seen here may be heightened in a more highly trained population, given the influence of sprint training on rates of peak glycolytic flux (Jacobs, Esbjörnsson, Sylvén, Holm, & Jansson, 1987). However, this would require further experimentation as it may be argued equally by contrast that such effects could be offset by the enhanced metabolic flexibility found with high intensity training (Perry, Heigenhauser, Bonen, & Spriet, 2008).

Significant effort was made in the present study to create a motivational climate that encouraged maximal performance from all participants during all trial efforts. The incentive scheme and group exercise dynamic were designed to increase motivation in contrast to the vast majority of nutritional ergogenic studies that ask participants to perform in isolation with little stimulation or external incentive (Buch et al., 2016). It seems likely therefore that the detrimental effects of nutritional ketosis seen here may have been minimised in comparison to single-participant study methods but should have greater ecological validity to the likely motivational climate found in

competitive sport (Atkinson & Nevill, 2001), where athletes are known to be more tolerant of issues such as GI distress for the benefit of their performance (Pfeiffer et al., 2012).

### **Conclusion**

Nutritional ketosis impaired repeat sprint cycling performance following pre-fatiguing exercise in a competitive group dynamic. Performance impairment with ketosis may have resulted from either or a combination of impaired peak exercise glycolytic flux and heightened gastro-intestinal discomfort, with the HPA axis appearing to play an important role in modulating the effects of nutritional ketosis on sprint performance. The utility of ketone ester feeding for sports performances with a significant requirement for repeated sprinting with short recovery is ill advised and the wider use of ketone ester feeding in mixed demand sports should be treated with caution.

## Chapter 7: The Utility of Ketone Ester Consumption in Sports Training and Competition: A Cross-Sport Case Study Series

### ABSTRACT

Evidence is growing for the ergogenic benefit of nutritional ketosis (Cox 2013; Cox et al., 2016) but little is known about the translation of this research into the sporting arena. This study aimed to describe the utility of ketone ester consumption in a series of sporting case studies and to examine the performance impact of nutritional ketosis in depth for a single sport. Ninety-one athletes across eight sports consumed a ketone ester drink (KET) at rest, during training and in competition, following individually tailored dosing regimens. Levels of ketosis following consumption were at the lower end of the previously published range (blood BHB:  $1.8 \pm 0.5$  mmol/l) with 40% of participants reporting mild and 41% reporting moderate gastro-intestinal (GI) symptoms. GI symptoms were more prevalent during training and competition ( $P < 0.01$ ) than at rest and 31% of participants withdrew from trialling due to the perceived side effects of the drink. In one team sport, a randomised cross-over trial comparing KET to a calorie matched carbohydrate drink (CHO) during tournament play demonstrated a reduction in low speed run distance ( $P < 0.05$ ) and an elevated GI symptom profile ( $P < 0.05$ ) with KET compared to CHO, alongside a trend towards an inverse relationship between GI symptom prevalence and high speed run distance independent of treatment ( $P = 0.09$ ). In contrast, subsequent self-selected ketone ester usage in high-level tournament play demonstrated an increase in high speed run distance ( $P < 0.05$ ) for those athletes who chose to maintain ketone ester usage versus no change for those who did not compared to a prior comparable tournament.

In conclusion, nutritional ketosis is achievable during sports training and competition but carries a GI symptom profile that is too problematic to warrant adherence for a significant number of athletes. For those athletes who chose to employ ketone ester consumption the performance impact remains equivocal.

## INTRODUCTION

Recently, an edible source of ketosis, in the form of a ketone ester, has been developed in an attempt to provide an alternative to ketogenic diets for Parkinson's disease and epilepsy (Kashiwaya et al., 2010). It is now possible to raise circulating ketone body concentration, with no acid load, in a dose dependent fashion by providing ketone esters in a drink, which can be consumed as part of a normal diet. This may offer similar benefits to the ketogenic diet, without the need for carbohydrate restriction. Since the ketone ester drink provides an alternative source of energy with potential benefits to metabolism, it is of great interest to investigate its role as an energy drink during exercise.

Early laboratory trials with ketone ester consumption indicate that nutritional ketosis can significantly alter substrate selection and exercise metabolism and may confer ergogenic benefit to highly trained athletes during sustained high intensity exercise of both steady and mixed demand (Cox, 2012; Cox et al., 2016). However, ketone ester dosing and drink tolerance (Chapter 4) are likely to be major mitigating factors in the search for ergogenic gain and there may be modes of exercise that can be adversely affected by ketone ester consumption (Chapter 6) warranting a degree of caution in the translation of laboratory findings into the field.

The practice of inferring the real world value of interventions aimed at improving sports performance, such as nutritional supplementation, by examining their impact on laboratory performance measures or physiological surrogates, has attracted

strong critique (Atkinson & Nevill, 2001). Whilst surrogate performance measures observed in highly controlled settings improve internal validity and increase the likelihood of a clear outcome, their translation to the externally valid world of elite sports performance is often questionable, particularly where a less highly trained population has been studied in an area where training status is likely to be a factor in the measured response. The difficulties inherent in gaining meaningful access to elite athletes has led some researchers to suggest that a more valid approach to establishing the efficacy of a sports performance intervention is to maximise the external validity of the research setting by examining athletes in their normal training or competition environment, whilst concurrently monitoring the many factors known to influence performance in this setting (T. B. Smith & Hopkins, 2011).

Recently, global positioning systems (GPS) have been used to objectively quantify movements in a range of team sports and have been shown to provide accurate and reliable measures of speed and distance covered (~5% coefficient of variation) during team sports performance (Coutts & Duffield, 2010; Jennings, Cormack, Coutts, Boyd, & Aughey, 2010; MacLeod, Morris, Nevill, & Sunderland, 2009; Portas, Harley, Barnes, & Rush, 2010). Furthermore, this technology is considered to be sufficiently valid and reliable to detect altered running across a match, between matches, between levels of competition, and between types of matches (Aughey, 2011).

GPS data from matches has been used previously to assess the efficacy of supplementation or dietary intervention (Minett, Duffield, & Bird, 2010). However, it is important to account for the match-to-match variability in activity profiles when

using GPS data from matches. Averaging match activity variables over multiple matches decreases the sample size required to detect a given effect (Aughey, 2011).

The present study aims to conduct a series of elite athlete case studies, over a wide competition window, to describe the impact of repeated dietary supplementation with a ketone ester on sports performances both in training and in competition. This study will take a two pronged approach utilising both a case study series and a more in depth single sport case study. Within the case study series (A) all participants will be tracked and characterised through a process of familiarisation with and utilisation of the ketone ester and asked to complete subjective ratings both of tolerance to the intervention and of perceived well-being whilst using the intervention during training and competition performances. The single sport case study (B) will employ a mixed methods approach with a combination of randomised, cross-over trialling of ketone ester use in competition and a descriptive comparison of the utility of ketone ester consumption in a normal competition setting, whilst making use of GPS tracking to characterise the impact on performance.

## **METHODS A – Descriptive Case Study Series**

### **Participants**

Ninety-one athletes from eight different sports participated in this study (n = 48 men: age  $25.7 \pm 3.9$  yr, height  $179.9 \pm 7.1$  cm, body mass (BM)  $75.5 \pm 8.5$  kg; n = 43 women: age  $25.1 \pm 5.7$  yr, height  $169.1 \pm 6.8$  cm, body mass (BM)  $59.7 \pm 7.5$  kg). All participants were active competitors at international level in their sport and the sports represented covered individual endurance sports (case studies A, D, E & G),

individual mixed demand sports (case studies B & H), team mixed demand sports (case study C) and team gameplay sports (case study F). The study received approval from the UK Sport Research Advisory Group and each participant was provided with a written and verbal briefing regarding the nature of the testing, and provided written, informed consent prior to participation.

### **Experimental Design**

This study involved a series of longitudinal, descriptive case studies across a number of both individual and team sports identified as having potential to benefit from nutritional ketosis. For each sporting case study, athletes were supported, in collaboration with appropriately trained personnel in each sport, through a process of experimenting with ketone ester consumption at rest, during training and in competition. Advice regarding ketone ester consumption was based upon emerging evidence for the factors influencing the tolerance and efficacy of nutritional ketosis (Cox, 2013; Chapters 3-6). For each descriptive case study blood ketone responses to treatment were characterised alongside subjective ratings of drink palatability, gastro-intestinal tolerance and affect, affording multiple opportunities to describe the effects of the ketone ester drink.

### **Experimental Protocol**

#### *Drink Formulation*

All ketone ester drinks were formulated, produced and secured in tamper proof bottles by an independent food specialist (IFN, Reading, UK) in accordance with



research guidance to provide a 1:1 caloric matching of ketone ester and carbohydrate from multiple sources (Chapter 2). Drinks were produced in shot form (75ml bottles containing a 32% solution of ketone ester and carbohydrate) and upon production were batch-tested for contaminants to ensure compliance with the World Anti-Doping Agency (WADA) code (Vouillamoz et al., 2009) and tracked through delivery to each case study sport where they remained in refrigerated storage until the bottle seal was broken by the athlete at the point of usage.

### *Preparatory Work*

For each case study, preliminary work (2-4 sessions) was conducted at rest and/or in training to familiarise participants to the ketone ester drink, assess individual tolerability and refine the dosing regimen to ensure the delivery of a sustained moderate ketosis during sporting performance. The development of optimal individual dosing always began with the ingestion of ketone ester at 8 mg/kg body mass/min of exercise, the dose shown to be most effective in previous trials for the induction of a sustained moderate ketosis. Dosing regimens were then refined individually on the basis of measured blood ketone concentration with a target range of 1.5-3.0mmol/l. Participants made up a dose from part or whole multiples of the 75ml shots and were encouraged to drink water *ad libitum* alongside dosing.

During preliminary testing and some further training and competition trials, where agreed with participants, capillary blood samples were collected prior to dosing, pre-exercise, during exercise and post-exercise for the analysis of blood ketone

concentration using portable handheld test meters (Optimum Xceed, Abbott Diabetes Care, Berkshire, UK).

### *Training and Competition Intervention*

For each case study, depending on perceptions of tolerance and efficacy during the preparatory work, participants were then offered repeated interventions with the ketone ester during training and competition performances. Participants were required to record subjective ratings of drink palatability and gastro-intestinal tolerance during all trials and encouraged where possible to record blood ketone concentration across the intervention period. Each case study sport was also encouraged to capture performance analysis data relating to interventional performances alongside any relevant co-factors for that sport (e.g. home advantage; technicality of course/opposition; concurrent nutritional and/or technical interventions) and where possible to complement this with historical performance data for comparison. However, with the exception of the trial data in section B of this study, performance data was not collated for reporting here.

### **Data Analysis**

Data from each sporting case study was characterised using descriptive statistics alongside overall responses across the case study series for comparison. Where appropriate, group differences were compared by one-way analysis of variance (ANOVA). All statistical analysis was conducted using IBM SPSS Statistics (Release

20.0.0, IBM, USA) and all data are presented as mean  $\pm$  SD. Statistical significance was set at the level of  $P < 0.05$ .

## RESULTS A – Descriptive Case Study Series

Usage of the ketone ester drink is summarised across all case studies in Table 7.1. Participants averaged around 4 interventions with the majority (58%) taking place in training and a significant number taking place in competition (36%). Blood ketone responses to ketone ester consumption in training and competition were on average at the lower end of the target range (average Blood BHB:  $1.8 \pm 0.5$ mmol/l) and drink pleasantness was consistently rated as moderately (45) or strongly (35) unpleasant (average pleasantness rating:  $41 \pm 8$ ).

	A	B	C	D	E	F	G	H	Overall
Participants	3	4	10	1	14	55	2	2	91
Total Usages	14	6	24	5	52	257	3	13	374
Ave Usage/Participant	4.7	1.5	2.4	5.0	3.7	4.7	1.5	6.5	4.1
Usage at rest			9		12				21
Usage in training	10	6	13	2	31	147	3	6	218
Usage in competition	4		2	3	9	110		7	135
Blood Ketone (mmol/l)	$1.5 \pm 0.4$	$1.4 \pm 0.4$	$1.9 \pm 1.0$	$2.0 \pm 0.5$	$2.5 \pm 1.2$	$1.5 \pm 0.7$	$1.4 \pm 0.7$	$1.8 \pm 1.0$	$1.8 \pm 0.5$
Drink pleasantness	$47 \pm 3$	$48 \pm 9$	$44 \pm 8$	$37 \pm 10$	$37 \pm 10$	$33 \pm 11$	$45 \pm 8$	$34 \pm 4$	$41 \pm 8$

Table 7.1. Treatment interventions at rest, in training and in competition characterised by participant and case study sport (A-H; n=91 athletes) alongside average blood ketone concentration and ratings of drink pleasantness (data are mean  $\pm$  SD).

The presence of gastro-intestinal (GI) symptoms (Table 7.2; Figure 7.1) was high across all trials with 40% of participants describing mild GI symptoms and 41% describing moderate GI symptoms. Whilst considerable variation in symptom

occurrence existed between case study sports, for participants who conducted resting trials there was a significant increase in mild and moderate GI symptoms during training and competition compared to trials at rest ( $P<0.01$ ).

	A	B	C	D	E	F	G	H	Overall
<b>All Sessions</b>									
% no GI symptoms	20%	50%	46%	100%	50%	11%	33%	20%	19%
% mild GI symptoms	30%	33%	29%	0%	36%	42%	67%	40%	40%
% moderate GI symptoms	50%	17%	25%	0%	14%	47%	0%	40%	41%
<b>Resting Trials</b>									
% no GI symptoms			67%		100%				80%
% mild GI symptoms			33%		0%				20%
% moderate GI symptoms			0%		0%				0%
<b>Training Trials</b>									
% no GI symptoms	13%	50%	38%	100%	29%	13%	33%	16%	16%
% mild GI symptoms	25%	33%	31%	0%	50%	39%	67%	38%	38%
% moderate GI symptoms	63%	17%	31%	0%	21%	48%	0%	46%	46%
<b>Competition Trials</b>									
% no GI symptoms	50%		0%	100%	44%	12%		43%	14%
% mild GI symptoms	50%		0%	0%	56%	41%		0%	41%
% moderate GI symptoms	0%		100%	0%	0%	47%		57%	46%

Table 7.2. Gastro-intestinal symptoms by case study sport at rest, during training and in competition (n=91 athletes; data are percentage of trial occurrences).

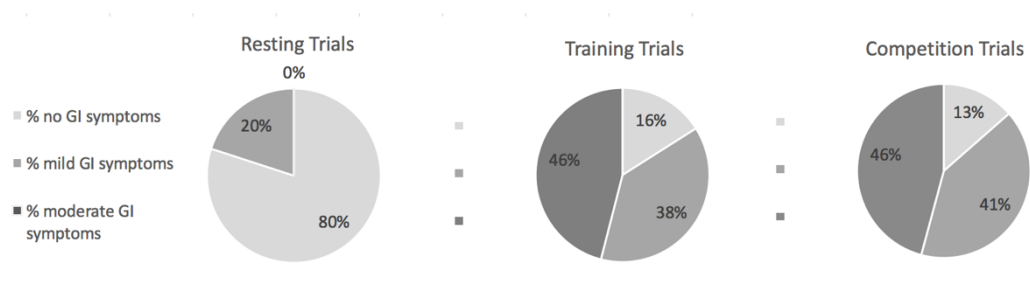


Figure 7.1. Gastro-intestinal symptoms across the case study series at rest, during training and in competition (n=91 athletes; data are percentage of trial occurrences).

### *Adherence*

Over the course of the trial period 28 athletes withdrew from trialling due to perceived side effects of the drink, 24 athletes withdrew from trialling due to a perceived lack of efficacy and 17 athletes withdrew from trialling due to lack of availability.

## **METHODS B – Single Sport Case Study**

### **Experimental Design**

Within one team sport case study a sub-cohort of participants (n = 15) engaged in a double-blinded, randomized, cross-over trial during tournament play followed by further unblinded competition trialling. During the cross-over trial each participant performed a minimum of three matches with at least one match following ketone ester consumption and at least one match following consumption of a control drink containing calorie matched carbohydrate. Drinks were consumed prior to warm-up and topped up again at half-time according to dosing regimens developed during preliminary work with this cohort (Methods A). Following the cross-over trial participants were free to choose whether to continue experimenting with the ketone ester drink unblinded in subsequent competitions.

### **Experimental Protocol**

All case study participants followed the guidelines in Methods A to become familiarised with the experimental drinks and develop optimal dosing regimens. Participants were instructed to record their food and fluid intake for 24 hours before the first match and to replicate this as closely as possible in the 24 hours before

subsequent matches. Participants were instructed to arrive for matches in a rested and fully hydrated state and had *ad libitum* access to water during all trials. Matches within tournament play were separated by 24 to 72 h.

### *Movement Tracking*

Participants were fitted with a GPS device (SPI Pro X, GPSports, Fyshwick, Australia) prior to each match and on-field activities were recorded by a single GPS unit for the duration of the game. GPS units were positioned between the scapular planes at T2-T6 of the spinal column and secured in place with a harness. Data from each GPS unit was immediately downloaded to a laptop computer and analysed using commercially available software (GPSports team AMS). Player movement variables were captured using GPS data and analysed for distances travelled during game time at different speeds, based on previously established speed zones in use by the case study sport.

### *Experimental Drinks*

Participants were randomly assigned drinks, match by match in a cross-over design, containing either a mixture of ketone ester and carbohydrate (in a 1:1 ratio) or a calorie matched carbohydrate only drink. Based upon ketone ester ingestion rates of 8mg/kg body mass/min of exercise, comparison carbohydrate feeding rates in the control drink fell into the range 0.8-1.2g/min of game time, depending on body mass. Drinks were delivered as a two-thirds bolus before the match and a one-third top-up bolus at half-time. Drinks were colour and flavour matched, as well as being matched for volume, with the support of an independent food technology specialist (IFN,

Reading, UK) and all drink formulation adhered to the processes described in Methods A.

### *Experimental Measures*

Participants rated the palatability of the both trial drinks based on a drink pleasantness scale (General Methods: Appendix 4) directly after consumption and were asked to rate the presence of any gastro-intestinal symptoms (General Methods: Appendix 5) post-consumption, pre-game and post-game.

Capillary blood samples were collected, where possible, prior to dosing, pre-exercise, during exercise and post-exercise for the analysis of blood ketone concentration using portable handheld test meters (Optimum Xceed, Abbott Diabetes Care, Berkshire, UK). Due to the demands of the competition environment blood sampling was requested rather than enforced.

### *Tournament Comparison*

Following the cross-over trial some participants chose to continue experimenting with the ketone ester drink in two subsequent tournaments and a prior tournament was included for comparison in the analysis. Tournaments 1 & 4 were conducted against a similar higher calibre of opposition and were therefore used to compare performance analysis data for participants who took part in both tournaments and completed Tournament 4 either with ( $n = 8$ ) or without ( $n = 7$ ) the ketone ester.

## Data Analysis

A paired two-tailed t test was used to identify differences between treatments, and a two-way (treatment x time) general liner model for repeated measures was employed for all serial measures, with the Greenhouse-Geisser correction applied where assumptions of sphericity were violated. Main effects were isolated by pairwise multiple comparisons using the Bonferroni method. All statistical analysis was conducted using IBM SPSS Statistics (Release 20.0.0, IBM, USA) and all data are presented as mean  $\pm$  SD. Statistical significance was set at the level of  $P < 0.05$ .

## RESULTS B – Single Sport Case Study

The blood ketone response to drink ingestion (Figure 7.2) demonstrated a consistent elevation of ketone body concentration across the exercise period following ketone ester ingestion compared to baseline ( $P < 0.001$ ) and compared to the ingestion of carbohydrate ( $P < 0.001$ ).

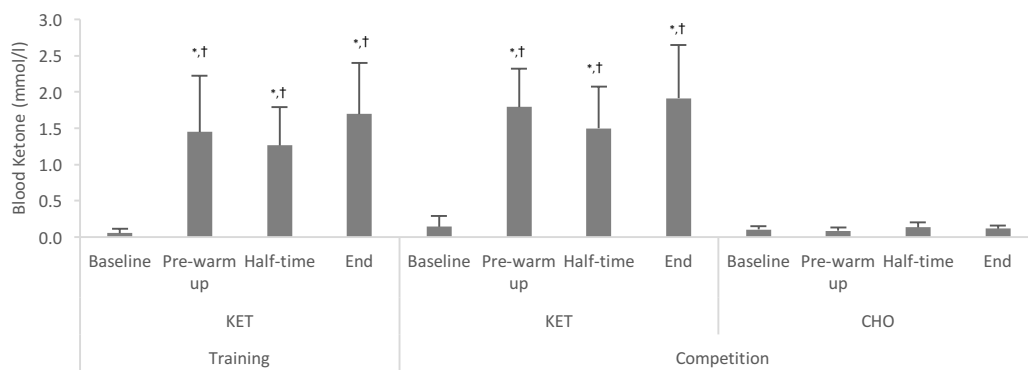


Figure 7.2. Blood ketone concentration during training and competition following ingestion of ketone ester (KET) or carbohydrate (CHO) drinks (n=15 athletes). Time-point versus baseline within treatment (\* =  $P < 0.001$ ); ketone ester versus carbohydrate within time-point († =  $P < 0.001$ ).



Gastro-intestinal symptom prevalence was high during training and competition following both ketone ester and carbohydrate consumption (Figure 7.3). There were no significant differences between treatments for mild symptom prevalence. However, prevalence of moderate symptoms was significantly higher following ketone ester consumption during both training ( $P < 0.05$ ) and competition ( $P < 0.05$ ) compared to carbohydrate consumption.

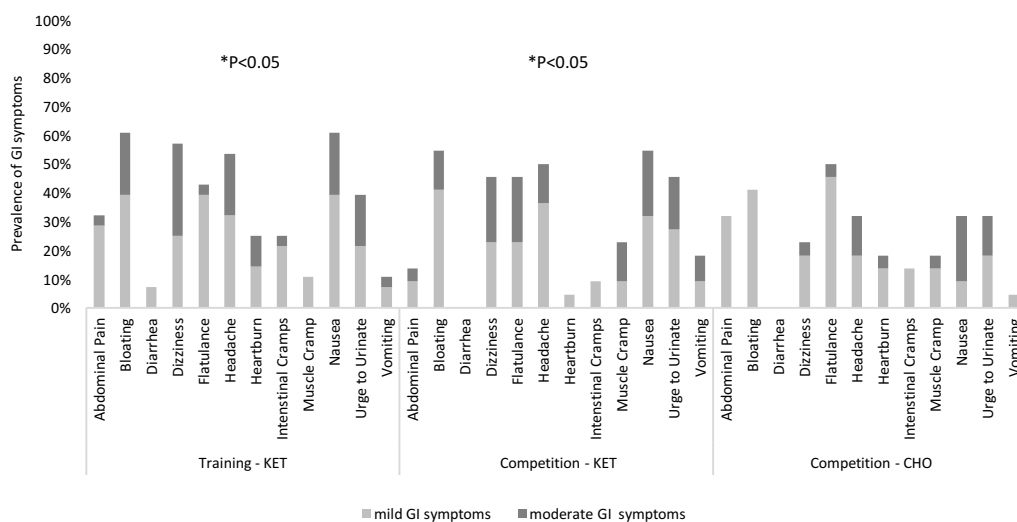


Figure 7.3. Gastro-intestinal symptom prevalence during training and competition following ingestion of ketone ester (KET) or carbohydrate (CHO) drinks (n=15 athletes). Moderate GI symptom treatment comparisons versus carbohydrate: \*  $P < 0.05$ .

### Competition Performance Analysis

There were no significant differences between tournaments for high speed distance run (Figure 7.4) although there were trends for lower high speed distances in Tournaments 2 & 3 compared to Tournaments 1 & 4 that did not reach significance.

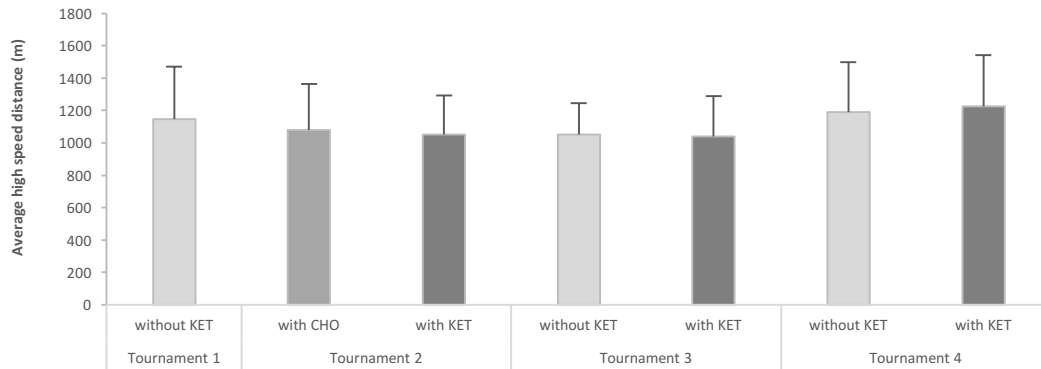


Figure 7.4. Average high speed distance run across four periods of tournament play with and without ketone ester (KET) or carbohydrate (CHO) ingestion (n=15 athletes; data are mean  $\pm$  SD).

### *Randomised, controlled Trial*

For the cross-over trial during Tournament 2 there were no significant differences in low speed run, high speed run or total distance covered between treatments when all participants were compared (Table 7.3). However, when only participants who had a treatment cross-over against the same opposition were compared there was a significantly lower low speed distance run ( $P < 0.05$ ) and a trend toward lower total distance which didn't reach significance ( $P = 0.09$ ) following consumption of ketone ester compared to carbohydrate.

		low speed run distance (m)	high speed run distance (m)	total Distance (m)
All matches (n=15)	Ketone Ester	3347.3 $\pm$ 560.0	1076.9 $\pm$ 231.7	6200.7 $\pm$ 802.5
	Carbohydrate	3345.2 $\pm$ 581.1	1117.4 $\pm$ 224.3	6051.9 $\pm$ 889.2
Opposition matches (n=7)	Ketone Ester	3225.1 $\pm$ 479.6*	1077.9 $\pm$ 264.6	5998.1 $\pm$ 753.1 <sup>†</sup>
	Carbohydrate	3533.4 $\pm$ 540.0	1129.4 $\pm$ 288.5	6340.1 $\pm$ 910.6

Table 7.3. low speed run, high speed run and total distance covered following consumption of ketone ester or carbohydrate in all matches (n=15 athletes) and only in matches with paired treatment comparison against the same opposition (n=7 athletes). Ketone ester versus carbohydrate: <sup>†</sup>  $P = 0.09$ ,

\*  $P < 0.05$ .

Independent of treatment condition there was a trend for high speed distance run to be lower when GI symptoms were moderate but this did not reach statistical significance (mild GI symptoms:  $1114.3 \pm 283.6\text{m}$  v moderate GI symptoms:  $993.3 \pm 218.6\text{m}$ ;  $P = 0.09$ ).

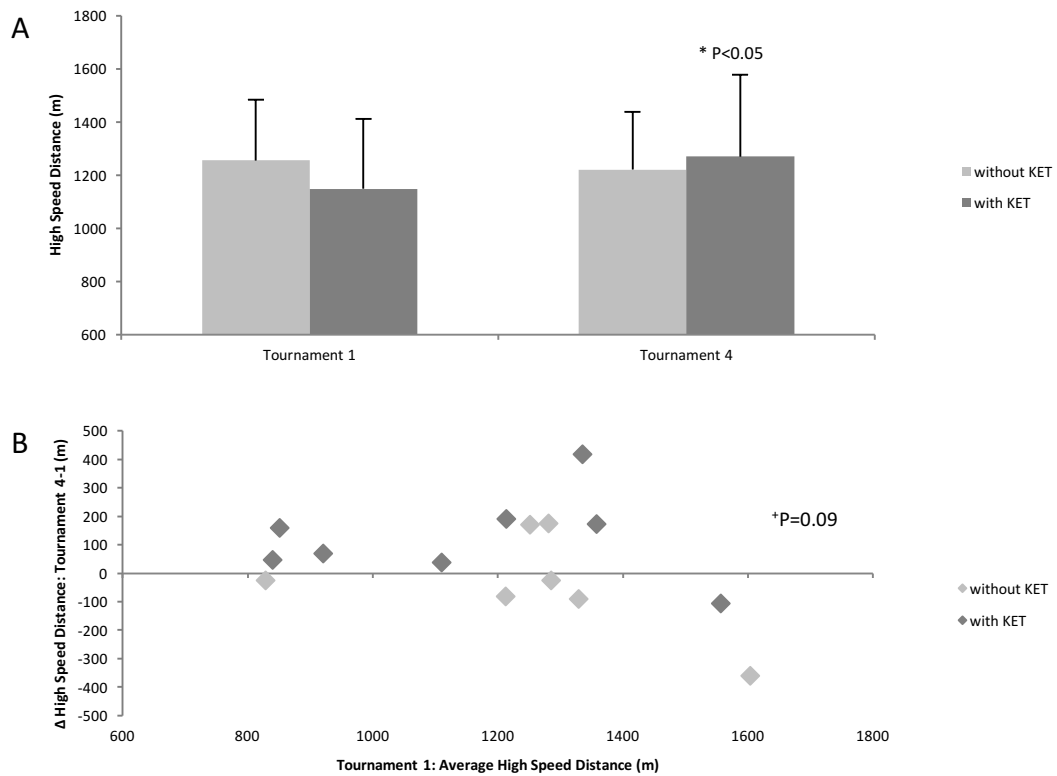


Figure 7.5. High speed run distances across two periods of tournament play for participants with (n=8 athletes; dark bars and diamonds) and without (n=7 athletes; light bars and diamonds) ketone ester (KET) consumption. A: Average high speed distance run at each tournament; Tournament 1 versus Tournament 4 within groups - \*  $P < 0.05$ . B: Average high speed distance run at Tournament 1 versus difference in distance run between Tournaments; with ketone ester versus without ketone ester - +  $P = 0.09$ .

### *Tournament Comparison*

There was a significant increase in high speed distance run from Tournament 1 to Tournament 4 for those participants who consumed ketone ester during Tournament 4 (Figure 7.5A;  $P < 0.05$ ) but no change for those participants who did not consume ketone ester. As a result, the difference between Tournament 4 and Tournament 1 for high speed distance run tended to be higher for ketone ester users versus non-users although this did not reach statistical significance (Figure 7.5B;  $P = 0.09$ ).

### **DISCUSSION**

The consumption of ketone ester drinks during real world sports training and competition results in a moderate nutritional ketosis that can be sustained in so far as drink palatability and gastric distress can be tolerated. Ketone ester drinks come with a significant side effect profile that is exaggerated by vigorous exercise and it is likely therefore that this novel nutritional intervention will not meet with widespread adoption in its current form.

Nutritional strategies for sport have long been known to offer somewhat of a poisoned chalice where perhaps fortune favours the brave. It is well established that well trained athletes can learn to tolerate the gastric discomfort associated with optimal carbohydrate ingestion for endurance exercise and in so doing confer the benefits of enhanced substrate supply for performance (Pfeiffer et al., 2012, 2009). However, it would appear here that the GI symptom profile associated with ketone ester consumption was somewhat worse than that for a calorie matched carbohydrate drink (Figure 7.3), suggesting a heightened risk of ketone ester usage.

Indeed, GI symptom prevalence was loosely correlated with performance decrement in a previous laboratory trial of ketone ester usage for repeat sprint performance (Chapter 6) suggesting that at least for some athletes in some contexts it may be foolhardy to bravely plough on through and tolerate worsening GI symptoms.

The optimal dosing of ketone esters for sports performance remains to be established in the majority of situations. Given the symptom profile described here alongside relatively low levels of ketosis relative to recent laboratory guidelines (Cox et al., 2016), and previous reports of increasing GI symptoms at higher doses of ketone ester even at rest (Clarke et al., 2012), it would appear that the promise of nutritional ketosis may be tempered, with the ketone ester in its current form, by a narrow window where efficacy meets tolerability. That three-quarters of participants in the current study withdrew themselves from further opportunities to experiment with the ketone ester suggest that this window may not be seen as viable for the majority of athletes.

The team sport case study reported here highlights both the promise and the perils of adoption of nutritional ketosis as a strategy for improvement of sports performance. Once the case study had progressed to the point where ketone ester adopters had continued by choice, which involved those with greater perceived tolerance issues self-selecting out, there was evidence of performance benefit with an increase in sustained high intensity running under the most demanding of competition settings (Figure 7.5). However, earlier in the study when participants were subject to the rigours of a blinded, randomised trial, ketone ester usage

appeared to reduce lower intensity run distances (Table 7.3), perhaps signalling a demotivating influence of the symptom profile.

Run distances in gameplay team sports do not confer ubiquitous advantage in all competitive contexts (Aughey, 2011). It is possible that, against a lower standard of opponent in Tournament 2 or simply a different kind of tactical challenge, participants simply employed adaptive strategies to win the game in ways other than utilising physical advantage by running faster and further than their opponents. Whether such advantages can be garnered when the opponent is of a higher capability is both unknown in the current context and a major confounding factor in general for attempts to assess the efficacy of discreet interventions during team sports performance. That significant differences were seen in run performance from players on the same team adopting alternate nutritional strategies provides both novel insight and further encouragement in pursuing an ecological approach (Phillips et al., 2010) to the assessment of interventional strategies for sports performance.

## **Conclusion**

Consumption of ketone ester drinks during real world sports training and performance resulted in moderate nutritional ketosis. Drinking ketone esters was generally considered unpleasant and resulted in a significant degree of gastric discomfort for the majority of athletes. The performance impact of nutritional ketosis was unclear with only limited evidence for benefit in high speed distance run during team sport where athletes had self-selected their participation based on prior experience with the consumption of the ketone ester. Whether nutritional ketosis can confer ergogenic benefit to athletes in a real-world sporting context remains

equivocal and appears to depend at least in part upon an individual ability to tolerate the drink.

## Chapter 8: General Discussion

### *Stress Physiology and Functional Dynamic Systems*

Since the march of early modern humans out of the African Savannah over 50,000 years ago (Stringer & Andrews, P., 1988), our genetic heritage has been inextricably linked with the competing demands of exploration and survival. The growing speculation, increasingly supported now by emerging archaeological and genetic evidence (Ingman, Kaessmann, Pääbo, & Gyllensten, 2000; White et al., 2003), that all modern humans can trace their lineage back to Africa, is testament to the enduring spirit of adventure which sits deep within us. It is this adventurous impulse which has driven us to scale improbable peaks, has fed our insatiable thirst for knowledge about the world around us and has led us to live longer, be stronger, travel further and communicate more widely than ever before. That such aspirational behaviour carries with it an inherent risk to our survival is at the very heart of the human condition.

*“But Mousie, thou art no thy lane,*

*In proving foresight may be vain :*

*The best-laid schemes o’ mice an’ men*

*Gang aft agley,*

*An’ lea’e us nought but grief an’ pain*

*For promis’d joy !*

*Still thou art blest, compared wi’ me !*



*The present only toucheth thee :  
But och ! I backward cast my e'e,  
On prospects drear !  
An' forward, tho' I canna see,  
I guess an' fear !"*

To a Mouse

On turning her up in her nest with the plough

(Robert Burns, 1785)

Whilst Burns may be forgiven for failing to foresee the discovery over two centuries later of the cognitive capacity of the murine neocortex to situationally dispose the mouse to exploit the memory of historical experience in the regulation of goal oriented behaviour (Dvorkin, Benjamini, & Golani, 2008), the view that existential awareness may be a uniquely human condition pervades despite significant attempts to identify a level of cognition in non-human primates suggestive of a systemic disposition to perceive beliefs and desires as master regulators of behavioural schema (Call & Tomasello, 2008). Nonetheless the core biological response to situational stress, be it born of plough or pity, remains a mainstay of mammalian physiology to which man is subject no less than mouse. Indeed, the cognitive capacity of humans to ponder existential anxieties, whilst being bound by evolutionary stress responses, may represent a particular point of pressure for human physiology with the potential to contribute in no small way to the maladies of modern life (Robert M.

Sapolsky, 2004). By way of example there is growing evidence that psychosocial stress can moderate the drive to eat and the selection of more palatable, energy dense food choices (Groesz et al., 2012). Such adaptations may have served the needs of our evolutionary forebears well when resources were scarce but have clear implications for the current global trends in weight gain and obesity seen in the presence of the food abundance more typical of modern post-agricultural life.

The concept of stress as a fundamental mediator of function was first introduced by Hans Selye in his “Studies on Adaptation” (Selye, 1937). Selye’s initial postulation was that each individual has a finite stock of ‘adaptation energy’ that can be drawn upon in response to the imposition of a stressor and further that the response to a wide variety of stressors is typified by a number of generic features that can be characterised by a ‘general adaptation reaction’, which proceeds through a temporal sequence of events described as the ‘stage of alarm’, the ‘stage of resilience’ and the ‘stage of exhaustion’. Selye himself furthered this view in the decade that followed by demonstrating that this stock of ‘adaptation energy’, whilst limiting in a dose dependent fashion to the short-term capacity for stress resilience, is in itself adaptable to the chronic effects of repeated stress (Selye, 1946).

A precise definition of stress has proved elusive in the intervening years but much work has been done to elaborate the notion that adaptive resilience can be traded up or down as a result of the manner in which the body is exposed to and deals with increases or decreases in stressful stimuli across the lifespan (McEwen, 2013). That stress can be harmful is well established but increasingly attention is being paid to

the potential for optimal amounts of stress to provoke desirable adaptations such that the relationship between stress and function may be best modelled by an inverted U dose response (Robert M. Sapolsky, 1997). For example, chronic psychosocial stress, in women caring for spouses with dementia, has been shown to predispose these women to oxidative damage during exposure to an acute stressor, whereas the same stressor applied to women with a low background of psychosocial stress results in an increased rather than decreased resilience to oxidative stress (Aschbacher et al., 2013). Such adaptive dynamics are at the heart of every response that the body mounts to a stressor. The response being determined not only by the degree of stimulus and the capacity of the system to act, but also by the concurrent needs of the organism as a whole and therefore willingness to act, the environmental factors at play and the memory of previous stressors, requiring the development of a wider view of adaptive behaviour as part of a dynamic functional system (Sudakov, 1997).

In his early experiments Selye recognised the critical importance of the endocrine system as a dynamic modulator of the functional response to stress exposure (Selye, 1937). The identification of extensive cross-talk between neural, immune and endocrine structures in the years that followed has further solidified the role of endocrine signals as functional regulators of resource deployment that are dynamically responsive to both mental and physical state and temporally responsive to historical experience. This is perhaps best exemplified by the cascade of events during illness that links elevations in inflammatory cytokines, through neuronal activation of the hypothalamic-pituitary-adrenal (HPA) axis, to a series of alterations

in systemic function and motivational state that combine to form sickness behaviours (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). The glucocorticoids (GC) in particular appear to be of critical importance in regulating both the metabolic and the behavioural response to acute and chronic stress.

Robert Sapolsky has gone far to elaborate, through his glucocorticoid cascade theory (R. M. Sapolsky, Krey, & McEwen, 1986), the many roles of the glucocorticoid signalling system in mobilising systemic resources and regulating behaviour in order to manage risk in the face of stressful stimuli. This extensive review established that acute upregulation of GC signalling is an essential component of the functional response to stress, with failures of the stress response occurring as a result of either an inability to activate glucocorticoid secretion in response to a stressor or an inability to terminate glucocorticoid secretion once the stress has subsided. In particular, prolonged chronic exposure to stress can result in a down-regulation of GC receptor sensitivity, requiring ever-higher increases in GC secretion to achieve the same level of functional response. Whilst acutely adaptive, ultimately this game of trading up can lead to a failure to maintain this upregulated function as energetic resources become compromised by the chronic heightening of demand (Angelier & Wingfield, 2012). Intriguingly, Sapolsky has demonstrated that enrichment of the fuel supply to the brain during such periods of heightened stress can enhance function and sustain stress resilience (R M Sapolsky, 1986). Further, it appears that this cerebral energetic compromise may be compounded by a GC cascade driven inhibition of uptake and utilisation of glucose since ketone ester feeding was more effective than glucose feeding in protecting against neuronal damage. The

behavioural corollary of this energetic cascade lies most pointedly in the sickness behaviours described above but it seems likely that more subtle perturbations in energetic load and stress resilience are at the heart of the challenge of coping with the day-to-day stresses of everyday life (Greenberg, Carr, & Summers, 2002).

A further complication of the adaptive dynamics of the stress response lies in the fact that the regulatory systems which have evolved to deal with stress are limited in their range, necessitating a specialisation of function at the local level from a common signal. Greenberg (Greenberg et al., 2002) has elaborated the view that situational stressors impinge not only on the processes directly challenged by the disturbance but also on a wide array of cofactors which are related to the same stress sensing and response pathways. Within the stress-adaptation cycle we therefore have a significant degree of feed-forward and feed-back cross talk creating a web of adaptation.

The myriad complexities of adaptive dynamics at the whole body level and the emergence of new technologies that allow an ever more powerful microscope to be fixed on the regulatory processes taking place within the cell have led a great many researchers to reduce their focus to the controllable dynamics of specific signalling systems at the cellular level with diminishing reference to the integration of micro and macro level system responses. In response to this trend a movement was started, some 20 years ago, which set out to defend the future of physiology against this march of reductionary thought. The prevailing view reported by the Commission on Bioengineering in Physiology at Glasgow's 32nd congress of the International Union

of Physiological Sciences (IUPS) was one of concern for the future of systems physiology. In 'The Logic of Life', a collection of essays that accompanied the congress programme, the tone was one of battle readiness (Noble & Boyd, 1993). That the debate rages on proves their foresight may not have been in vain (Greenhaff & Hargreaves, 2011). The establishment and growth of the physiome project, born of the Glasgow battle cry, is an encouraging early sign of the emergence of a maturing view of an internal environment that achieves "integration from proteins to organs" (Hunter, Smith, Fernandez, & Tawhai, 2005). In expanding upon this view of intelligent, integrated functional systems, future physiological research would do well to consider that the brain appears, through the collation of historical experiences, situational stressors and relative risk, to manage the dynamics of functional adaptation in much the same way as it manages the delivery of a single bout of exercise, through an anticipatory model that weighs both risk and opportunity to manage behaviour (Noakes, 2008).

Exercise as a model of stress physiology offers a great many advantages since each acute stressor can be accurately characterised by the regulation of external work and the well-trained athlete provides a highly controllable experimental framework for examining the adaptive characteristics of stress exposure. When examining adaptation to exercise training and sports performance from the perspective of dynamic functional systems (Phillips et al., 2010) it therefore becomes necessary to give due consideration to such factors as the training history of experimental participants, the concurrent training stresses imposed during the experimental condition and the likelihood of a behavioural conflict between the desired outcome

and the resources requiring to be mobilised in order to achieve it. That such information is seldom reported in the literature on exercise training and sports performance is perhaps indicative of the extent to which the debate has become wedded to reductionary thought.

It is becoming increasingly popular to examine the acute cellular environment following a single exercise stimulus, with an ever more sophisticated microscope being applied to the transient perturbations evident in Bernard's 'milieu intérieur' (Holmes, 1986). At this juncture, Bernard himself has sent forward some prophetic words: "It is of the greatest importance to consider the influence of the nervous system on the chemical phenomena of the organs, for it is by this influence that the living being is in contact with everything, and everything can then act upon it. There is the true terrain of the influence of mind over matter."

#### *Nutritional Ketosis and Sports Performance*

The work reported here aimed to take a holistic view of the utility of a novel nutritional intervention for sports performance from the perspective of the behavioural biology at play. This work was conducted in conjunction with a parallel research programme that focussed on the energetic mechanisms underpinning the potential ergogenic benefit of nutritional ketosis (Cox, 2012; Cox et al., 2016). As a result, this thesis focusses largely upon the ecological validity of this ongoing research in relation to its translation onto the sports field. There are myriad complexities in the functional response to nutritional ketosis both at the level of physiological effect and in relation to functional impact. What appears superficially to be a simple issue

of substrate supply and energetics is not even half of the picture given the psychodynamics at play when an athlete chooses to adopt a nutritional intervention in the cauldron of the sporting arena.

The findings presented here sound a largely cautionary note and go some way to describing the bounds of efficacy for utility of the ketone ester drink. That nutritional ketosis can significantly alter exercising substrate selection and energetics and in so doing may confer a small but significant positive impact on endurance exercise performance in well trained athletes (Cox et al., 2016) is of interest for sports performance. The focus of the work reported here was to begin exploring the limits of this nutritional state and the practicalities of ketotic sports performance in order that athletes may make better informed choices regarding the adoption of this novel nutritional intervention.

In the field of sports nutrition it is all too common for limited laboratory findings to be translated well beyond their limits and used to promote commercial and competitive interests without full consideration of the ecological validity of the research findings (Atkinson & Nevill, 2001; Lundberg et al., 2011). With public interest in nutritional ketosis accelerating more rapidly than the available evidence, the basis for employing ketone ester consumption for sports performance has been questioned (Pinckaers et al., 2016). In consideration of these concerns and in order to address the ecological dynamics at play, the findings presented here highlight the practical factors influencing ketone ester dosing (Chapters 3 & 7), the importance of optimal dosing within tolerable constraints (Chapters 4, 6 & 7), the modes and



contexts of exercise helped by nutritional ketosis and perhaps more importantly those with potential to be harmed (Chapters 4, 6 & 7) and the psycho-physiological dynamics modulating the behavioural response to ketotic exercise performance (Chapters 5 & 6).

Whilst fraught with practical challenges the field based case studies utilised here (Chapter 7) were a critical aspect of elucidating the real world impact of ketone ester consumption. That athletes were able to adopt practical dosing strategies (Chapter 3) leading to sustained ketosis during competitive sports performance (Chapter 7: Figure 7.2) and that, for some, this may have resulted in performance benefit (Chapter 7: Figure 7.5A) is of great significance. However, this must be tempered with equivocal evidence for wider performance impact (Chapter 7: Table 7.3) alongside the realisation that gastro-intestinal symptoms were highly prevalent in the competitive arena (Chapter 7: Figure 7.3) and for a large number of athletes (31%) were too much to bear. It is possible that athletes can build up a tolerance to GI symptoms in order to sustain the benefits of nutritional intervention (Pfeiffer et al., 2012) but it seems likely that the symptom profile of ketone ester consumption will remain a deterrent to usage for some.

Given the high individual variation in symptom reporting, it would be of significant interest to explore the factors underpinning symptom prevalence. It is not currently known whether symptom prevalence may be altered by alternate vehicles for nutritional ketosis. Whilst it appears likely that ketone salts (Plecko et al., 2002) and other ketogenic agents, such as medium chain triglycerides (Jeukendrup, Thielen,

Wagenmakers, Brouns, & Saris, 1998), will fail to deliver an adequate ketotic stimulus in tolerable doses, it remains possible that the development of alternate ketone esters may dampen the symptom profile.

Work presented here indicates that there may be a lower threshold of benefit for nutritional ketosis given the absence of impact of low dose ketone ester consumption (6mg/min/kg-body-mass) on either markers of glycolytic metabolism (Chapter 4: Figure 4.3) or cycling time trial performance, in contrast to work presented elsewhere under similar conditions but at a higher ketone ester dose (Cox et al., 2016). Given the GI symptom profile associated with higher levels of dosing (Chapter 6) and the aggravated symptom profile seen for ketone ester usage in the field (Chapter 7: Figure 7.3) it would appear that there may be a narrow window of efficacy regarding optimal ketone ester dosing for sports performance. GI symptoms were significantly elevated following the higher dose of ketone ester (10mg/min/kg-body-mass) employed in the repeat sprint cycling trial reported here (Chapter 6: Table 6.3). The extent to which the symptom profile was responsible for the decrement in sprint performance with ketosis is unclear, but this level of symptom prevalence points to a heightened risk of employing higher levels of dosing during high intensity exercise.

Perhaps the most important finding reported here was the negative impact of ketone ester consumption on repeat sprint cycling performance following a pre-fatiguing bout of exercise (Chapter 6: Figure 6.1). This clearly defines not only the bounds of efficacy of nutritional ketosis but highlights the fact that in the absence of ergogenic benefit ketone ester consumption may be far from inert in its effects. Whilst the

prevalence of GI symptoms may have played a role in this response, it appears likely that the very metabolic effects that may confer benefit during more sustained endurance exercise (Cox et al., 2016) may cause harm during more intensive levels of demand. In particular, the intramuscular suppression of glycolysis and enhancement of lipolysis seen by Cox et al. following ketone ester consumption was hypothesised to offer a favourable energetic state supporting the observed improvement in 30-minute cycling time trial performance following 60 minutes of pre-fatiguing exercise. By contrast the suppression of peak plasma lactate in the data reported here (Chapter 6: Table 6.1) during repeat sprint exercise may have negated the ability of participants to optimise the energetic backdrop for sprint performance. Performance impairment occurred in a task requiring the maintenance of intermittent sprinting which accumulated five minutes of high intensity effort within a twenty-minute period following twenty minutes of moderate intensity pre-fatiguing work. This brings into question the benefit of nutritional ketosis for shorter endurance events that involve regular excursions into sustained high intensity effort (Sharma et al., 2015).

Another key finding reported here was the relationship between the dynamics of HPA axis activity and the performance outcome of the repeat sprint trial (Chapter 6: Figure 6.2). Emerging awareness of the role of androgenic and glucocorticoid signalling in the regulation of competitive behaviour represents a new frontier in behavioural biology (McEwen, 2013) that could go some way to bridging the gap between holistic and mechanistic approaches to understanding the dynamics of the stress response. In updating Bernard's earlier conception of 'mind over matter' it

would appear that a more appropriate frame of reference for exploring the dynamics of human behaviour would be to take a psycho-neuro-endocrinological perspective on emergent behaviour framed by an awareness of the ecological dynamics at play in influencing both the short and long term sequence of events (Phillips et al., 2010). That a modest correlation was found here between performance outcome and testosterone/cortisol ratio signalling is not in and of itself of particular note. However, that this relationship emerged against the backdrop of a competitive motivational climate (Corbett et al., 2012b) which encouraged both performance and mastery goals (Pensgaard & Roberts, 2000) goes some small way towards highlighting the inherently complex dynamics influencing the behaviour of study participants invited to give of their best in the hope that their efforts will confer some wider meaning beyond their own circumstances.

#### *Future Recommendations for Research on Novel Nutritional Interventions*

In the rush to seek benefit, both competitive and commercial, from ergogenic novelty there is often an unholy collusion between provider and recipient of new research findings, with the ensuing promise of a future bestowed that most often betrays the deeper subtleties at play. Whilst it will never be possible to research all possible eventualities of the application of scientific research, in the field of sports performance ergogenics it would appear that much could be done to bridge the gap between research and practice.

There is a great deal that could be done within the laboratory setting to more clearly identify and attend to the array of factors that influence both trial performance and

its translation into the field. Moving beyond a cursory distinction between trained and untrained participants, and towards a more nuanced understanding of participant characteristics would be welcome. Efforts in this direction should consider more fully the training and performance history of experimental participants as well as their motivational orientation in relation to the task at hand. Perhaps most importantly, laboratory performance trials would do well to seek creative ways of enhancing the motivational climate for performance trialling in ways that more effectively reflect the motivation climate of sports performance.

The advantages of field based case study approaches are significant, particularly where more direct access to the target population can be sought. Greater efforts to conduct experimental work on NNIs in the field would likely hasten the journey to impact and highlight issues of translation that could improve both the quality and communication of work across domains. Whilst there are aspects of due diligence in preparing a case for NNI adoption based on sound laboratory science, it is likely that future innovations will move towards a rapid prototyping approach that shortens the distance between research and practice. Such initiatives will require the development of novel experimental approaches in order to be effective.

The future of nutritional ketosis for sports performance is clearly a promising one. This novel nutritional state imparts profound effects on exercising metabolism and profoundly challenges our historical views on substrate selection and energetics. However, care should be taken not to rush to broad brush conclusions regarding the benefits of performing in a ketotic state. Much remains to be elucidated in

elaborating the situations within which ketosis may confer favourable advantage to function. As things stand there is room to be cautiously optimistic whilst perhaps avoiding the promise of 'best laid plans'.

## REFERENCES

- Abbiss, C. R., & Laursen, P. B. (2008). Describing and understanding pacing strategies during athletic competition. *Sports Medicine*, *38*(3), 239–252.  
<http://doi.org/10.2165/00007256-200838030-00004>
- Angelier, F., & Wingfield, J. C. (2012). Importance of the glucocorticoid stress response in a changing world: Theory, hypotheses and perspectives. *General and Comparative Endocrinology*, *190*(September), 118–128.  
<http://doi.org/10.1016/j.ygcen.2013.05.022>
- Ansley, L., Robson, P. J., St. Clair Gibson, A., & Noakes, T. T. D. (2004). Anticipatory Pacing Strategies during Supramaximal Exercise Lasting Longer than 30 s. *Medicine and Science in Sports and Exercise*, *36*(2), 309–314.  
<http://doi.org/10.1249/01.MSS.0000113474.31529.C6>
- Aschbacher, K., O'Donovan, A., Wolkowitz, O. M., Dhabhar, F. S., Su, Y., & Epel, E. (2013). Good stress, bad stress and oxidative stress: Insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology*, *38*(9), 1698–1708.  
<http://doi.org/10.1016/j.psyneuen.2013.02.004>
- Atkinson, G., & Nevill, A. M. (2001). Selected issues in the design and analysis of sport performance research. *Journal of Sports Sciences*, *19*(10), 811–827.  
<http://doi.org/10.1080/026404101317015447>
- Atkinson, G., Peacock, O., St Clair Gibson, A., & Tucker, R. (2007). Distribution of Power Output During Cycling. *Sports Medicine*, *37*(8), pp.647-667.  
<http://doi.org/10.2165/00007256-200737080-00001>
- Aughey, R. J. (2011). Applications of GPS technologies to field sports. *International Journal of Sports Physiology and Performance*, *6*(3), 295–310.

<http://doi.org/10.1080/02640414.2013.868920>

Bailey, S. J., Vanhatalo, A., Wilkerson, D. P., Dimenna, F. J., & Jones, A. M. (2009).

Optimizing the “priming” effect: influence of prior exercise intensity and recovery duration on O<sub>2</sub> uptake kinetics and severe-intensity exercise tolerance. *Journal of Affective Disorders*, *107*(10), 1743–1756.

<http://doi.org/00810.2009> [pii]\r10.1152/jappphysiol.00810.2009

Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R., Dimenna, F. J., Wilkerson, D.

P., ... Jones, A. M. (2009). Dietary nitrate supplementation reduces the O<sub>2</sub> cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Affective Disorders*, *107*(6), 1144–1155.

<http://doi.org/10.1152/jappphysiol.00722.2009>.—Pharmacological

Bartoshuk, L. M., Duffy, V. B., Green, B. G., Hoffman, H. J., Ko, C. W., Lucchina, L. A.,

... Weiffenbach, J. M. (2004). Valid across-group comparisons with labeled scales: The gLMS versus magnitude matching. *Physiology and Behavior*, *82*(1),

109–114. <http://doi.org/10.1016/j.physbeh.2004.02.033>

Beaven, C. M., Maulder, P., Pooley, A., Kilduff, L., & Cook, C. (2013). Effects of

caffeine and carbohydrate mouth rinses on repeated sprint performance.

*Applied Physiology, Nutrition, and Metabolism*, *38*(6), 633–7.

<http://doi.org/10.1139/apnm-2012-0333>

Beneke, R., & Boning, D. (2008). The limits of human performance. *Essays in*

*Biochemistry*, *44*, 11–26. <http://doi.org/10.1042/BSE0440011>

Bergström, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, Muscle Glycogen

and Physical Performance. *Acta Physiologica Scandinavica*, *71*(2–3), 140–150.

<http://doi.org/10.1111/j.1748-1716.1967.tb03720.x>



- Bescós, R., Ferrer-Roca, V., Galilea, P. A., Roig, A., Drobnic, F., Sureda, A., ... Pons, A. (2012). Sodium nitrate supplementation does not enhance performance of endurance athletes. *Medicine and Science in Sports and Exercise*, 44(12), 2400–2409. <http://doi.org/10.1249/MSS.0b013e3182687e5c>
- Bickham, D. C., Bentley, D. J., Le Rossignol, P. F., & Cameron-Smith, D. (2006). The effects of short-term sprint training on MCT expression in moderately endurance-trained runners. *European Journal of Applied Physiology*, 96(6), 636–643. <http://doi.org/10.1007/s00421-005-0100-x>
- Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitation Medicine*, 2–3, 92–98.
- Bosch, A. N., Dennis, S. C., & Noakes, T. D. (1994). Influence of carbohydrate ingestion on fuel substrate turnover and oxidation during prolonged exercise. *Journal of Applied Physiology*, 76(6), 2364–2372.
- Boushel, R. (2017). Linking skeletal muscle blood flow and metabolism to the limits of human performance. *Applied Physiology, Nutrition, and Metabolism*, 42, 111–115.
- Brooks, G. A. (2002). Lactate shuttles in nature. *Biochemical Society Transactions*, 30(2), 258–64. <http://doi.org/10.1042/>
- Brooks, G. A. (2007). Lactate: link between glycolytic and oxidative metabolism. *Sports Medicine*, 37(4–5), 341–343. <http://doi.org/10.2165/00007256-200737040-00017>
- Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *Journal of Physiology*, 587(Pt 23), 5591–600. <http://doi.org/10.1113/jphysiol.2009.178350>

- Brooks, G. A., Dubouchaud, H., Brown, M., Sicurello, J. P., & Butz, C. E. (1999). Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proceedings of the National Academy of Sciences*, 96(3), 1129–1134. <http://doi.org/10.1073/pnas.96.3.1129>
- Buch, R., Nerstad, C. G., Aandstad, A., & Säfvenbom, R. (2016). Exploring the interplay between the motivational climate and goal orientation in predicting maximal oxygen uptake. *Journal of Sports Sciences*, 34(3), 267–277. <http://doi.org/10.1080/02640414.2015.1048522>
- Burd, N. a, Tang, J. E., Moore, D. R., & Phillips, S. M. (2009). Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. *Journal of Applied Physiology*, 106(5), 1692–701. <http://doi.org/10.1152/jappphysiol.91351.2008>
- Burke, L. M., Hawley, J. A., Wong, S. H. S., & Jeukendrup, A. E. (2011). Carbohydrates for training and competition. *Journal of Sports Sciences*, 29 Suppl 1(April), S17-27. <http://doi.org/10.1080/02640414.2011.585473>
- Burnett, R. W., & Noonan, D. C. (1974). Calculations and correction factors used in determination of blood pH and blood gases. *Clinical Chemistry*, 20(12), 1499–1506.
- Byrne, H. a, Tieszen, K. L., Hollis, S., Dornan, T. L., & New, J. P. (2000). Evaluation of an electrochemical sensor for measuring blood ketones. *Diabetes Care*, 23(4), 500–503. <http://doi.org/10.2337/diacare.23.4.500>
- Cahill, G. F., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., ... Kipnis, D. M. (1966). Hormone-fuel interrelationships during fasting. *Journal of Clinical Investigation*, 45(11), 1751–1769. <http://doi.org/10.1172/JCI105481>

- Cairns, S. P. (2006). Lactic acid and exercise performance: Culprit or friend? *Sports Medicine*, 36(4), 279–291. <http://doi.org/10.2165/00007256-200636040-00001>
- Call, J., & Tomasello, M. (2008). Does the chimpanzee have a theory of mind? 30 years later. *Trends in Cognitive Sciences*, 12(5), 187–192. <http://doi.org/10.1016/j.tics.2008.02.010>
- Carter, J. M., Jeukendrup, A. E., & Jones, D. A. (2004). The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Medicine and Science in Sports and Exercise*, 36(12), 2107–2111. <http://doi.org/10.1249/01.MSS.0000147585.65709.6F>
- Chatham, J. C. (2002). Lactate - the forgotten fuel! *Journal of Physiology*, 542(2), 333–333. <http://doi.org/10.1113/jphysiol.2002.020974>
- Christensen, P. M., Nyberg, M., & Bangsbo, J. (2013). Influence of nitrate supplementation on VO<sub>2</sub> kinetics and endurance of elite cyclists. *Scandinavian Journal of Medicine and Science in Sports*, 23(1), 1–11. <http://doi.org/10.1111/sms.12005>
- Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., Todd King, M., Musa-Veloso, K., ... Veech, R. L. (2012). Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regulatory Toxicology and Pharmacology*, 63(3), 401–408. <http://doi.org/10.1016/j.yrtph.2012.04.008>
- Clarkson, P. M., Volek, J. S., Kraemer, W. J., Rubin, M. R., Gómez, A. L., Ratamess, N. a., ... Nakhostin-Roohi, B. (2002). New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *American Journal of Physiology. Endocrinology and Metabolism*, 5(2), 7.

<http://doi.org/10.1152/ajpendo.00277.2001>

Coff, C. (1983). Incorporation, Digestion and Incarnation. *History*, 55(1), 1–9.

<http://doi.org/10.1016/j.aqpro.2013.07.003>

Cook, C. J., & Crewther, B. T. (2012a). Changes in salivary testosterone concentrations and subsequent voluntary squat performance following the presentation of short video clips. *Hormones and Behavior*, 61(1), 17–22.

<http://doi.org/10.1016/j.yhbeh.2011.09.006>

Cook, C. J., & Crewther, B. T. (2012b). The effects of different pre-game motivational interventions on athlete free hormonal state and subsequent performance in professional rugby union matches. *Physiology and Behavior*, 106(5), 683–688. <http://doi.org/10.1016/j.physbeh.2012.05.009>

Cook, C. J., Crewther, B. T., & Smith, A. A. (2012). Comparison of baseline free testosterone and cortisol concentrations between elite and non-elite female athletes. *American Journal of Human Biology*, 24(6), 856–858.

<http://doi.org/10.1002/ajhb.22302>

Coombes, J. S., & Hamilton, K. L. (2000). The effectiveness of commercially available sports drinks. *Sports Medicine*, 29(3), 181–209.

<http://doi.org/10.2165/00007256-200029030-00004>

Corbett, J., Barwood, M. J., Ouzounoglou, A., Thelwell, R., & Dicks, M. (2012a). Influence of competition on performance and pacing during cycling exercise. *Medicine and Science in Sports and Exercise*, 44(3), 509–515.

<http://doi.org/10.1249/MSS.0b013e31823378b1>

Corbett, J., Barwood, M. J., Ouzounoglou, A., Thelwell, R., & Dicks, M. (2012b). Influence of competition on performance and pacing during cycling exercise.

*Medicine and Science in Sports and Exercise*, 44(3), 509–515.

<http://doi.org/10.1249/MSS.0b013e31823378b1>

Cordain, L. et al. (2002). The paradoxical nature of hunter-gatherer diets: meat-based, yet non-atherogenic. *European Journal of Clinical Nutrition*, 56(Suppl 1), S42–S52. <http://doi.org/10.1038/sj.ejcn.1601353>

Coutts, A. J., & Duffield, R. (2010). Validity and reliability of GPS devices for measuring movement demands of team sports. *Journal of Science and Medicine in Sport*, 13(1), 133–135. <http://doi.org/10.1016/j.jsams.2008.09.015>

Cox, P. (2012). *The effects of a novel substrate on exercise energetics in elite athletes*. University of Oxford.

Cox, P., & Clarke, K. (2014). Acute nutritional ketosis: implications for exercise performance and metabolism. *Extreme Physiology & Medicine*, 3, 17. <http://doi.org/10.1186/2046-7648-3-17>

Cox, P., Kirk, T., Ashmore, T., Willerton, K., Evans, R., Smith, A., ... Clarke, K. (2016). Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. *Cell Metabolism*, 24(2), 256–268. <http://doi.org/10.1016/j.cmet.2016.07.010>

Crewther, B. T., Cook, C., Cardinale, M., Weatherby, R. P., & Lowe, T. (2011). Two Emerging Concepts for Elite Athletes Neuromuscular System and the Dose-Response Training Role of these Endogenous Hormones. *Sports Medicine*, 41(2), 103–123. <http://doi.org/10.2165/11539170-000000000-00000>

Currell, K., & Jeukendrup, A. E. (2008). Superior endurance performance with ingestion of multiple transportable carbohydrates. *Medicine and Science in Sports and Exercise*, 40(2), 275–281.

<http://doi.org/10.1249/mss.0b013e31815adf19>

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008).

From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews. Neuroscience*, *9*(1), 46–56.

<http://doi.org/10.1038/nrn2297>

Desrochers, S., Quiuze, K., Dugas, H., Dubreuil, P., Bomont, C., David, F., ...

Brunengraber, H. (1995). Potential Alternates To Lipid Emulsions for Total Parenteral Nutrition. *Synthesis*, *2863*(94), 111–118.

Driller, M. W., Argus, C. K., & Shing, C. M. (2013). The reliability of a 30-s sprint test on the wattbike cycle ergometer. *International Journal of Sports Physiology and Performance*, *8*(4), 379–383.

Dvorkin, A., Benjamini, Y., & Golani, I. (2008). Mouse cognition-related behavior in the open-field: Emergence of places of attraction. *PLoS Computational Biology*, *4*(2), e1000027. <http://doi.org/10.1371/journal.pcbi.1000027>

Dymsza, H. A. (1975). Nutritional application and implication of 1,3 butanediol. *Federation Proceedings*, *34*(12), 2167–2170.

Emhoff, C. W., Messonnier, L. A., Horning, M. A., Fattor, J. A., Carlson, T. J., & Brooks, G. A. (2013). Direct and indirect lactate oxidation in trained and untrained men. *Journal of Applied Physiology*, *115*(6), 829–38. <http://doi.org/10.1152/jappphysiol.00538.2013>

Fernström, M., Tonkonogi, M., & Sahlin, K. (2004). Effects of acute and chronic endurance exercise on mitochondrial uncoupling in human skeletal muscle. *Journal of Physiology*, *554*(Pt 3), 755–763.

<http://doi.org/10.1113/jphysiol.2003.055202>

- Fontaine, E., & Leverve, X. M. (2001). Role of Substrates in the Regulation of Mitochondrial Function In Situ. *IUBMB Life*, 52(3–5), 221–229.  
<http://doi.org/10.1080/15216540152846037>
- Frye, G. D., Chapin, R. E., Vogel, R. A., Mailman, R. B., Kilts, C. D., Mueller, R. A., & Breese, G. R. (1981). Effects of acute and chronic 1,3-butanediol treatment on central nervous system function: A comparison with ethanol. *Journal of Pharmacology and Experimental Therapeutics*, 216(2), 306–314.
- Gibala, M. J., Little, J. P., van Essen, M., Wilkin, G. P., Burgomaster, K. a, Safdar, A., ... Tarnopolsky, M. a. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *Journal of Physiology*, 575(Pt 3), 901–911.  
<http://doi.org/10.1113/jphysiol.2006.112094>
- Gonzalez, J. T., Veasey, R. C., Rumbold, P. L. S., & Stevenson, E. J. (2013). Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *British Journal of Nutrition*, 110(4), 721–32.  
<http://doi.org/10.1017/S0007114512005582>
- Greenberg, N., Carr, J. a, & Summers, C. H. (2002). Causes and Consequences of Stress. *Integrative and Comparative Biology*, 42(3), 508–516.  
<http://doi.org/10.1093/icb/42.3.508>
- Greenhaff, P. L., & Hargreaves, M. (2011). “Systems biology” in human exercise physiology: is it something different from integrative physiology? *Journal of Physiology*, 589(Pt 5), 1031–1036.  
<http://doi.org/10.1113/jphysiol.2010.201525>
- Groesz, L. M., McCoy, S., Carl, J., Saslow, L., Stewart, J., Adler, N., ... Epel, E. (2012).

What is eating you? Stress and the drive to eat. *Appetite*, 58(2), 717–721.

<http://doi.org/10.1016/j.appet.2011.11.028>

Guy, J. H., Deakin, G. B., Edwards, A. M., Miller, C. M., & Pyne, D. B. (2015).

Adaptation to Hot Environmental Conditions: An Exploration of the Performance Basis, Procedures and Future Directions to Optimise Opportunities for Elite Athletes. *Sports Medicine*, 45(3), 303–311.

<http://doi.org/10.1007/s40279-014-0277-4>

Hakkinen, K., & Pakarinen, A. (1995). Acute hormonal responses to heavy resistance

exercise in men and women at different ages. *International Journal of Sports Medicine*, 16(8), 507–513. <http://doi.org/10.1055/s-2007-973045>

Hardy, C., & Rejeski, W. (1989). Not what, but how one feels: the measurement of affect during exercise. *Journal of Sport & Exercise Psychology*, 11, 304–317.

<http://doi.org/10.1123/jsep.11.3.304>

Hargreaves, M., Hawley, J. A., & Jeukendrup, A. (2004). Pre-exercise carbohydrate

and fat ingestion: effects on metabolism and performance. *Journal of Sports Sciences*, 22(1), 31–38. <http://doi.org/10.1080/0264041031000140536>

Hashim, S. a, & Vanitallie, T. B. (2014). Ketone body therapy: from ketogenic diet to oral administration of ketone ester. *Journal of Lipid Research*, 55(9), 1818–26.

<http://doi.org/10.1194/jlr.R046599>

Hawley, J. A., & Burke, L. M. (1997). Effect of meal frequency and timing on physical performance. *British Journal of Nutrition*, 77(S1), S91.

<http://doi.org/10.1079/BJN19970107>

Hayes, L. D., Bickerstaff, G. F., & Baker, J. S. (2010). Interactions of Cortisol,

Testosterone, and Resistance Training: Influence of Circadian Rhythms.



*Chronobiology International*, 27(4), 675–705.

<http://doi.org/10.3109/07420521003778773>

Hermansen, L., Hultman, E., & Saltin, B. (1967). Muscle Glycogen during Prolonged Severe Exercise. *Acta Physiologica Scandinavica*, 71(2–3), 129–139.

<http://doi.org/10.1111/j.1748-1716.1967.tb03719.x>

Hickson, R. C., Rennie, M. J., Conlee, R. K., Winder, W. W., & Holloszy, J. O. (1977). Effects of increased plasma fatty acids on glycogen utilization and endurance. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 43(5), 829–833.

Holmes, L. M. (1986). Claude Bernard, the “milieu intérieur”, and regulatory physiology. *History & Philosophy of the Life Sciences*, 8(1), 3–25.

<http://doi.org/10.2307/23328847>

Horner, N. K., Patterson, R. E., Neuhouser, M. L., Lampe, J. W., Beresford, S. A., & Prentice, R. L. (2002). Participant characteristics associated with errors in self-reported energy intake from the Women’s Health Initiative food-frequency questionnaire. *American Journal of Clinical Nutrition*, 76(4), 766–773.

<http://doi.org/10.1096/fj.10-171983>

Hunter, P., Smith, N., Fernandez, J., & Tawhai, M. (2005). Integration from proteins to organs: The IUPS Physiome Project. *Mechanisms of Ageing and Development*, 126(1), 187–192. <http://doi.org/10.1016/j.mad.2004.09.025>

Ingman, M., Kaessmann, H., Pääbo, S., & Gyllensten, U. (2000). Mitochondrial genome variation and the origin of modern humans. *Nature*, 408(6813), 708–713. <http://doi.org/10.1038/35047064>

Jabre, M. G., & Bejjani, B. P. W. (2006). Treatment of Parkinson disease with diet-

induced hyperketonemia: A feasibility study [13]. *Neurology*, 66(4), 617.

<http://doi.org/10.1212/01.wnl.0000216108.57529.b1>

Jacobs, I., Esbjornsson, M., Sylven, C., Holm, I., & Jansson, E. (1987). Sprint training effects on muscle myoglobin, enzymes, fiber types, and blood lactate.

*Medicine and Science in Sports and Exercise*, 19(4), 368–374.

<http://doi.org/10.1249/00005768-198708000-00008>

Jacobs, I., Tesch, P. A., Karlsson, J., & Dotan, R. (1983). Lactate in human skeletal muscle after 10 and 30 s of supramaximal exercise. *Journal of Applied Physiology*, 55(2), 365–367.

*Physiology*, 55(2), 365–367.

Jennings, D., Cormack, S., Coutts, A. J., Boyd, L. J., & Aughey, R. J. (2010). Variability of GPS units for measuring distance in team sport movements. *International Journal of Sports Physiology and Performance*, 5(4), 565–569.

<http://doi.org/10.1123/ijsp.2014-0294>

Jeukendrup, A. E. (2004). Carbohydrate intake during exercise and performance.

*Nutrition*, 20(7–8), 669–677. <http://doi.org/10.1016/j.nut.2004.04.017>

Jeukendrup, A. E., & Killer, S. C. (2011). The myths surrounding pre-exercise carbohydrate feeding. *Annals of Nutrition and Metabolism*, 57(SUPPL. 2), 18–25. <http://doi.org/10.1159/000322698>

Jeukendrup, A. E., Thielen, J. J. H. C., Wagenmakers, A. J. M., Brouns, F., & Saris, W. H. M. (1998). Effect of medium-chain triacylglycerol and carbohydrate ingestion during exercise on substrate utilization and subsequent cycling performance. *American Journal of Clinical Nutrition*, 67(3), 397–404.

Joyner, M. J., & Coyle, E. F. (2008). Endurance exercise performance: the physiology of champions. *Journal of Physiology*, 586(1), 35–44.

<http://doi.org/10.1113/jphysiol.2007.143834>

Joyner, M. J., & Saltin, B. (2008). Exercise physiology and human performance: systems biology before systems biology! *Journal of Physiology*, *586*(1), 9.

<http://doi.org/10.1113/jphysiol.2007.148411>

Kashiwaya, Y., Bergman, C., Lee, J. H., Wan, R., King, M. T., Mughal, M. R., ... Veech, R. L. (2013). A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. *Neurobiology of Aging*, *34*(6), 1530–1539.

<http://doi.org/10.1016/j.neurobiolaging.2012.11.023>

Kashiwaya, Y., King, M. T., & Veech, R. L. (1997). Substrate signaling by insulin: A ketone bodies ratio mimics insulin action in heart. *American Journal of Cardiology*, *80*(3 A), 50A–64A. [http://doi.org/10.1016/S0002-9149\(97\)00458-X](http://doi.org/10.1016/S0002-9149(97)00458-X)

Kashiwaya, Y., Pawlosky, R., Markis, W., King, M. T., Bergman, C., Srivastava, S., ... Veech, R. L. (2010). A ketone ester diet increases brain malonyl-CoA and uncoupling proteins 4 and 5 while decreasing food intake in the normal Wistar rat. *Journal of Biological Chemistry*, *285*(34), 25950–25956.

<http://doi.org/10.1074/jbc.M110.138198>

Kelley, D. E., & Mandarino, L. J. (2000). Fuel selection in human skeletal muscle in insulin resistance: A reexamination. *Diabetes*, *49*(5), 677–683.

<http://doi.org/10.2337/diabetes.49.5.677>

Kiens, B., Essen-Gustavsson, B., Christensen, N. J., & Saltin, B. (1993). Skeletal muscle substrate utilization during submaximal exercise in man: effect of endurance training. *Journal of Physiology*, *469*, 459–78.

<http://doi.org/10.1113/jphysiol.1993.sp019823>

- Kitabchi, A. E., Umpierrez, G. E., Murphy, M. B., & Kreisberg, R. A. (2006).  
Hyperglycemic crises in adult patients with diabetes: A consensus statement  
from the American Diabetes Association. *Diabetes Care*, 29(12), 2739–2748.  
<http://doi.org/10.2337/dc06-9916>
- Koeslag, J. H. (1982). Post exercise ketosis and the hormone response to exercise: a  
review. *Medicine and Science in Sports and Exercise*, 14(5), 327–334.
- Koeslag, J. H., Noakes, T. D., & Sloan, A. W. (1980). Post-exercise ketosis. *Journal of  
Physiology*, 301, 79–90.
- Kossoff, E. H. (2004). More fat and fewer seizures: Dietary therapies for epilepsy.  
*Lancet Neurology*, 3(7), 415–420. [http://doi.org/10.1016/S1474-  
4422\(04\)00807-5](http://doi.org/10.1016/S1474-4422(04)00807-5)
- Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to  
resistance exercise and training. *Sports Medicine*, 35(4), 339–361.  
<http://doi.org/10.2165/00007256-200535040-00004>
- Kwiterovich, Jr, P. O., Vining, E. P. G., Pyzik, P., Richard Skolasky, J., Freeman, J. M.,  
PA, S., ... MJ, K. (2003). Effect of a High-Fat Ketogenic Diet on Plasma Levels of  
Lipids, Lipoproteins, and Apolipoproteins in Children. *JAMA : The Journal of the  
American Medical Association*, 290(7), 912.  
<http://doi.org/10.1001/jama.290.7.912>
- L. Veech, Britton Chance, Yoshihiro, R. (2001). Ketone Bodies, Potential Therapeutic  
Uses. *IUBMB Life*, 51(4), 241–247.  
<http://doi.org/10.1080/152165401753311780>
- Lansley, K. E., Winyard, P. G., Bailey, S. J., Vanhatalo, A., Wilkerson, D. P., Blackwell,  
J. R., ... Jones, A. M. (2011). Acute dietary nitrate supplementation improves

cycling time trial performance. *Medicine and Science in Sports and Exercise*, 43(6), 1125–1131. <http://doi.org/10.1249/MSS.0b013e31821597b4>

Lansley, K. E., Winyard, P. G., Fulford, J., Vanhatalo, A., Bailey, S. J., Blackwell, J. R., ... Dietary, J. a M. (2011). Dietary nitrate supplementation reduces the O<sub>2</sub> cost of walking and running: a placebo-controlled study. *Journal of Applied Physiology*, 110(3), 591–600. <http://doi.org/jappphysiol.01070.2010> [pii];10.1152/jappphysiol.01070.2010 [doi]

Lundberg, J. O., Larsen, F. J., & Weitzberg, E. (2011). Supplementation with nitrate and nitrite salts in exercise: a word of caution. *Journal of Applied Physiology*, 111(2), 616–617. <http://doi.org/10.1152/jappphysiol.00521.2011>

MacLeod, H., Morris, J., Nevill, A., & Sunderland, C. (2009). The validity of a non-differential global positioning system for assessing player movement patterns in field hockey. *Journal of Sports Sciences*, 27(2), 121–128. <http://doi.org/10.1080/02640410802422181>

Marwood, S., Constantin-Teodosiu, D., Casey, E., Whyte, M., Boobis, L., & Bowtell, J. (2010). No acetyl group deficit is evident at the onset of exercise at 90% of maximal oxygen uptake in humans. *Journal of Sports Sciences*, 28(3), 267–279. <http://doi.org/10.1080/02640410903440884>

McEwen, B. S. (2013). Correction for McEwen, Brain on stress: How the social environment gets under the skin. *Proceedings of the National Academy of Sciences*, 110(4), 1561.2-1561. <http://doi.org/10.1073/pnas.1221399110>

McNally, M. A., Pyzik, P. L., Rubenstein, J. E., Hamdy, R. F., & Kossoff, E. H. (2009). Empiric Use of Potassium Citrate Reduces Kidney-Stone Incidence With the Ketogenic Diet. *Pediatrics*, 124(2), e300–e304.

<http://doi.org/10.1542/peds.2009-0217>

Mikkelsen, K. H., Seifert, T., Secher, N. H., Grondal, T., & Van Hall, G. (2015).

Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-β-hydroxybutyratemia in post-absorptive healthy males. *Journal of Clinical Endocrinology and Metabolism*, *100*(2), 636–643.

<http://doi.org/10.1210/jc.2014-2608>

Minett, G., Duffield, R., & Bird, S. P. (2010). Effects of acute multinutrient supplementation on rugby union game performance and recovery.

*International Journal of Sports Physiology and Performance*, *5*(1), 27–41.

<http://doi.org/10.1016/j.jsams.2009.10.058>

Mitchell, J. B., Costill, D. L., Houmard, J. A., Fink, W. J., Pascoe, D. D., & Pearson, D.

R. (1989). Influence of carbohydrate dosage on exercise performance and glycogen metabolism. *Journal of Affective Disorders*, *67*(5), 1843–1849.

Molé, P. a, & Hoffmann, J. J. (1999). VO<sub>2</sub> kinetics of mild exercise are altered by

RER. *Journal of Applied Physiology*, *87*(6), 2097–2106.

Noakes, T. D. (2008). Testing for maximum oxygen consumption has produced a

brainless model of human exercise performance. *British Journal of Sports Medicine*, *42*(7), 551–5. <http://doi.org/10.1136/bjism.2008.046821>

Noble, D., & Boyd, C. A. R. (1993). *The challenge of integrative physiology*. (C. Boyd

& D. Noble, Eds.) *The logic of life*. Oxford University Press.

Ormsbee, M. J., Bach, C. W., & Baur, D. A. (2014). Pre-exercise nutrition: The role of

macronutrients, modified starches and supplements on metabolism and endurance performance. *Nutrients*, *6*(5), 1782–1808.

<http://doi.org/10.3390/nu6051782>

- Peacock, O., Tjønnå, A. E., James, P., Wisløff, U., Welde, B., Böhlke, N., ... Sandbakk, Ø. (2012). Dietary nitrate does not enhance running performance in elite cross-country skiers. *Medicine and Science in Sports and Exercise*, 44(11), 2213–2219. <http://doi.org/10.1249/MSS.0b013e3182640f48>
- Pensgaard, a M., & Roberts, G. C. (2000). The relationship between motivational climate, perceived ability and sources of distress among elite athletes. *Journal of Sports Sciences*, 18(3), 191–200. <http://doi.org/10.1080/026404100365090>
- Perry, C. G. R., Heigenhauser, G. J. F., Bonen, A., & Spriet, L. L. (2008). High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. *Applied Physiology, Nutrition, and Metabolism*, 33(6), 1112–1123. <http://doi.org/10.1139/H08-097>
- Pfeiffer, B., Cotterill, A., Grathwohl, D., Stellingwerff, T., & Jeukendrup, A. E. (2009). The effect of carbohydrate gels on gastrointestinal tolerance during a 16-km run. *International Journal of Sport Nutrition and Exercise Metabolism*, 19(5), 485–503.
- Pfeiffer, B., Stellingwerff, T., Hodgson, A. B., Randell, R., Pöttgen, K., Res, P., & Jeukendrup, A. E. (2012). Nutritional intake and gastrointestinal problems during competitive endurance events. *Medicine and Science in Sports and Exercise*, 44(2), 344–351. <http://doi.org/10.1249/MSS.0b013e31822dc809>
- Phillips, E., Davids, K., Renshaw, I., & Portus, M. (2010). Expert performance in sport and the dynamics of talent development. *Sports Medicine*, 40(4), 271–283. <http://doi.org/10.2165/11319430-000000000-00000>
- Pinckaers, P. J. M., Churchward-Venne, T. A., Bailey, D., & van Loon, L. J. C. (2016). Ketone Bodies and Exercise Performance: The Next Magic Bullet or Merely

Hype? *Sports Medicine*, 1–9. <http://doi.org/10.1007/s40279-016-0577-y>

Pitsiladis, Y. P., Smith, I., & Maughan, R. J. (1999). Increased fat availability enhances the capacity of trained individuals to perform prolonged exercise.

*Medicine and Science in Sports and Exercise*, 31(11), 1570–9.

<http://doi.org/10.1097/00005768-199911000-00012>

Plecko, B., Stoeckler-Ipsiroglu, S., Schober, E., Harrer, G., Mlynarik, V., Gruber, S., ...

Ipsiroglu, O. (2002). Oral b-Hydroxybutyrate Supplementation in Two Patients with Hyperinsulinemic Hypoglycemia: Monitoring of b-Hydroxybutyrate Levels in Blood and Cerebrospinal Fluid, and in the Brain by In Vivo. *Pediatric Research*, 52(2), 301–306.

*Pediatric Research*, 52(2), 301–306.

<http://doi.org/10.1023/01.PDR.0000019439.27135.2B>

Poplawski, M. M., Mastaitis, J. W., Isoda, F., Grosjean, F., Zheng, F., & Mobbs, C. V.

(2011). Reversal of diabetic nephropathy by a ketogenic diet. *PLoS ONE*, 6(4), e18604. <http://doi.org/10.1371/journal.pone.0018604>

Portas, M. D., Harley, J. A., Barnes, C. A., & Rush, C. J. (2010). The validity and

reliability of 1-Hz and 5-Hz Global Positioning Systems for linear, multidirectional, and soccer-specific activities. *International Journal of Sports Physiology and Performance*, 5(4), 448–458.

<http://doi.org/10.1123/ijsp.5.4.448>

Puchowicz, M. A., Smith, C. L., Bomont, C., Koshy, J., David, F., & Brunengraber, H.

(2000). Dog model of therapeutic ketosis induced by oral administration of R,S-1,3-butanediol diacetoacetate. *Journal of Nutritional Biochemistry*, 11(5), 281–

287. [http://doi.org/10.1016/S0955-2863\(00\)00079-6](http://doi.org/10.1016/S0955-2863(00)00079-6)

Randle, P. J. (1998). Regulatory interactions between lipids and carbohydrates: The



glucose fatty acid cycle after 35 years. *Diabetes/Metabolism Reviews*, 14(4), 263–283. [http://doi.org/10.1002/\(SICI\)1099-0895\(199812\)14:4<263::AID-DMR233>3.0.CO;2-C](http://doi.org/10.1002/(SICI)1099-0895(199812)14:4<263::AID-DMR233>3.0.CO;2-C)

Randle, P. J., Garland, P. B., Hales, C. N., & Newsholme, E. A. (1963). The Glucose Fatty-Acid Cycle Its Role in Insulin Sensitivity and the Metabolic Disturbances of Diabetes Mellitus. *The Lancet*, 281(7285), 785–789. [http://doi.org/10.1016/S0140-6736\(63\)91500-9](http://doi.org/10.1016/S0140-6736(63)91500-9)

Rasmussen, P., Wyss, M. T., & Lundby, C. (2011). Cerebral glucose and lactate consumption during cerebral activation by physical activity in humans. *FASEB Journal*, 25(9), 2865–2873. <http://doi.org/10.1096/fj.11-183822>

Rauch, H. G. L., St Clair Gibson, a, Lambert, E. V., & Noakes, T. D. (2005). A signalling role for muscle glycogen in the regulation of pace during prolonged exercise. *British Journal of Sports Medicine*, 39(1), 34–38. <http://doi.org/10.1136/bjism.2003.010645>

Renfree, A., Martin, L., Richards, A., St, A., & Gibson, C. (2012). All for One and One for All ! Disparity Between Overall Crew ' s and Individual Rowers ' Pacing Strategies During Rowing. *International Journal of Sports Physiology and Performance*, 7(Cv), 1–4. <http://doi.org/10.1139/h03-041>

Rolfe, D. F., & Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological Reviews*, 77(3), 731–58.

Rolls, B. (2000). The role of energy density in overconsumption of fat. *Journal of Nutrition*, 130(2S), 272–275.

Romijn, J. a, Coyle, E. F., Sidossis, L. S., Rosenblatt, J., & Wolfe, R. R. (2000).

Substrate metabolism during different exercise intensities in endurance-

trained women. *Journal of Applied Physiology*, 88(5), 1707–1714.

[http://doi.org/10.1016/s0026-0495\(96\)90016-5](http://doi.org/10.1016/s0026-0495(96)90016-5)

Romijn, J., Gastaldelli, A., Horowitz, J., Endert, E., & Wolfe, R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology. Endocrinology and Metabolism*, 265(3p1), 380–391.

Rose, A. J., & Richter, E. A. (2005). Skeletal muscle glucose uptake during exercise: how is it regulated? *Physiology*, 20(August 2016), 260–270.

<http://doi.org/10.1152/physiol.00012.2005>

Sapolsky, R. M. (1986). Glucocorticoid toxicity in the hippocampus: reversal by supplementation with brain fuels. *Journal of Neuroscience*, 6(8), 2240–2244.

Sapolsky, R. M. (1997). McEwen-Induced Modulation of Endocrine History: A Partial Review. *Stress*, 2(1), 1–11. <http://doi.org/10.3109/10253899709014733>

Sapolsky, R. M. (2004). *Why zebras don't get ulcers. Natural History* (Third Edit, Vol. 79). St Martin's Press. <http://doi.org/10.1086/425775>

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrine Reviews*, 7(3), 284–301. <http://doi.org/10.1210/edrv-7-3-284>

Schrauwen, P., Troost, F. J., Xia, J., Ravussin, E., & Saris, W. H. (1999). Skeletal muscle UCP2 and UCP3 expression in trained and untrained male subjects. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity*, 23(9), 966–72. <http://doi.org/10.1038/sj.ijo.0801026>

Schussel, H. (1953). Sparing effect of polyhydric alcohols in nutrition, and some

remarks on enlarging the basis of our nutrition. *Klinische Wochenschrift*, 31, 768.

Selye, H. (1937). Studies on adaptation. *Endocrinology*, 21(2), 169–188.

<http://doi.org/10.1210/endo-21-2-169>

Selye, H. (1946). Citation classics: The general adaptation syndrome and the diseases of adaptation. *Journal of Clinical Endocrinology*, 6(13), 117–231.

<http://doi.org/10.1016/j.ajog.2010.07.025>

Sharma, A. P., Elliott, A. D., & Bentley, D. J. (2015). Reliability and validity of a new variable-power performance test in road cyclists. *International Journal of Sports Physiology and Performance*, 10(3), 278–284.

<http://doi.org/10.1123/ijsp.2014-0013>

Smith, A. a, Toone, R., Peacock, O., Drawer, S., Stokes, K. a, & Cook, C. J. (2013). Dihydrotestosterone is elevated following sprint exercise in healthy young men. *Journal of Applied Physiology*, 114(March 2013), 1435–40.

<http://doi.org/10.1152/jap.2012.114.3.1435>

Smith, T. B., & Hopkins, W. G. (2011). Variability and predictability of finals times of elite rowers. *Medicine and Science in Sports and Exercise*, 43(11), 2155–2160.

<http://doi.org/10.1249/MSS.0b013e31821d3f8e>

Stringer Andrews, P., C. B. (1988). Genetic and fossil evidence for the origin of modern humans. *Science*, 239(4845), 1263–1268.

Sudakov, K. V. (1997). The theory of functional systems: general postulates and principles of dynamic organization (dedicated to the Anokhin Centenary).

*Integrative Physiological and Behavioral Science : The Official Journal of the Pavlovian Society*, 32(4), 392–414. <http://doi.org/10.1007/BF02688634>

Tatterson, A. J., Hahn, A. G., Martini, D. T., & Febbraio, M. A. (2000). Effects of heat stress on physiological responses and exercise performance in elite cyclists.

*Journal of Science and Medicine in Sport*, 3(2), 186–193.

[http://doi.org/10.1016/S1440-2440\(00\)80080-8](http://doi.org/10.1016/S1440-2440(00)80080-8)

Thomas, K., Elmeua, M., Howatson, G., & Goodall, S. (2016). Intensity-Dependent Contribution of Neuromuscular Fatigue after Constant-Load Cycling. *Medicine and Science in Sports and Exercise*, 48(9), 1751–1760.

<http://doi.org/10.1249/MSS.0000000000000950>

Timmons, J. A., Gustafsson, T., Sundberg, C. J., Jansson, E., Hultman, E., Kaijser, L., ... Greenhaff, P. L. (1998). Substrate availability limits human skeletal muscle oxidative ATP regeneration at the onset of ischemic exercise. *Journal of Clinical Investigation*, 101(1), 79–85. <http://doi.org/10.1172/JCI1146>

Tucker, R. (2009). The anticipatory regulation of performance: the physiological basis for pacing strategies and the development of a perception-based model for exercise performance. *British Journal of Sports Medicine*, 43(6), 392–400. <http://doi.org/10.1136/bjism.2008.050799>

Tucker, R., & Noakes, T. D. (2009). The physiological regulation of pacing strategy during exercise: a critical review. *British Journal of Sports Medicine*, 43(6), e1–e1. <http://doi.org/10.1136/bjism.2009.057562>

van Hall, G., Strömstad, M., Rasmussen, P., Jans, O., Zaar, M., Gam, C., ... Nielsen, H. B. (2009). Blood lactate is an important energy source for the human brain. *Journal of Cerebral Blood Flow & Metabolism*, 29(6), 1121–1129.

<http://doi.org/10.1038/jcbfm.2009.35>

Van Loon, L. J. C., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H. M., &

- Wagenmakers, A. J. M. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *Journal of Physiology*, 536(1), 295–304. <http://doi.org/10.1111/j.1469-7793.2001.00295.x>
- Vouillamoz, M., Thom, C., Grisdale, R., Saugy, M., Giraud, S., Robinson, N., ... Geisendorfer, T. (2009). *Anti-doping testing at the 2008 European football championship. Drug Testing and Analysis* (Vol. 1).
- Vukovich, M. D., Costill, D. L., Hickey, M. S., Trappe, S. W., Cole, K. J., & Fink, W. J. (1993). Effect of fat emulsion infusion and fat feeding on muscle glycogen utilization during cycle exercise. *Journal of Affective Disorders*, 75(4), 1513–1518.
- White, T. D., Asfaw, B., DeGusta, D., Gilbert, H., Richards, G. D., Suwa, G., & Howell, F. C. (2003). Pleistocene *Homo sapiens* from Middle Awash, Ethiopia. *Nature*, 423(6941), 742–747. <http://doi.org/10.1038/nature01669>
- Wooles, A., Robinson, A., & Keen, P. (2005). A static method for obtaining a calibration factor for SRM bicycle power cranks. *Sports Engineering*, 8(August 2016), 137–144. <http://doi.org/10.1007/BF02844014>
- Yancy, W. S., Foy, M., Chalecki, A. M., Vernon, M. C., Westman, E. C., & Westman, E. C. (2005). A low-carbohydrate, ketogenic diet to treat type 2 diabetes. *Nutrition & Metabolism*, 2, 34. <http://doi.org/10.1186/1743-7075-2-34>
- Zavorsky, G. S., Cao, J., Mayo, N. E., Gabbay, R., & Murias, J. M. (2007). Arterial versus capillary blood gases: A meta-analysis. *Respiratory Physiology and Neurobiology*, 155(3), 268–279. <http://doi.org/10.1016/j.resp.2006.07.002>
- Zhang, Y., Huo, M., Zhou, J., & Xie, S. (2010). PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel.

*Computer Methods and Programs in Biomedicine*, 99(3), 306–314.

<http://doi.org/10.1016/j.cmpb.2010.01.007>

## APPENDICES

### Appendix 1

#### Training History Questionnaire

This questionnaire is designed to gather information on your training history to give us more information on the volume and intensity of your training throughout your lifetime. All data collected will be treated confidentially.

---

Name

These next questions refer to all of your sporting activities. If you were previously more highly trained, please fill out questions for both your current and former athletic status.

	<b>CURRENT</b>	<b>FORMER</b>
How would you describe your athletic status?	<input type="checkbox"/> Non Sporting <input type="checkbox"/> Recreational athlete <input type="checkbox"/> Top Level/ Competitive athlete	<input type="checkbox"/> Non Sporting <input type="checkbox"/> Recreational athlete <input type="checkbox"/> Top Level/ Competitive athlete
How long have you been exercising on a regular basis?	<input type="checkbox"/> Since Childhood <input type="checkbox"/> Years <input type="checkbox"/> Do not exercise regularly	
If you were previously more highly trained, how long ago was this? And for how long did you train at this level?	...	...
How many times do you/did you exercise per week?	...	...
Are you/were you an active member of a sports team/club?	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No

Which sports currently form/previously formed a regular part of your training? (tick all that apply)

- |   |   |
|---|---|
| <input type="checkbox"/> Ball/Team Games                          | <input type="checkbox"/> Ball/Team Games                          |
| <input type="checkbox"/> Resistance training                      | <input type="checkbox"/> Resistance training                      |
| <input type="checkbox"/> Running                                  | <input type="checkbox"/> Running                                  |
| <input type="checkbox"/> Cycling                                  | <input type="checkbox"/> Cycling                                  |
| <input type="checkbox"/> Martial Arts                             | <input type="checkbox"/> Martial Arts                             |
| <input type="checkbox"/> Fitness classes (please provide details) | <input type="checkbox"/> Fitness classes (please provide details) |
| <input type="checkbox"/> Swimming                                 | <input type="checkbox"/> Swimming                                 |
| <input type="checkbox"/> Triathlon                                | <input type="checkbox"/> Triathlon                                |
| <input type="checkbox"/> Gymnastics/Dance                         | <input type="checkbox"/> Gymnastics/Dance                         |
| <input type="checkbox"/> Walking                                  | <input type="checkbox"/> Walking                                  |
| <input type="checkbox"/> Other (please provide details)           | <input type="checkbox"/> Other (please provide details)           |

If you regularly take part/took part in resistance training, which forms of training do/did you do?

- |  |  |
|--|--|
| <input type="checkbox"/> Strength training | <input type="checkbox"/> Strength training |
| <input type="checkbox"/> Power training    | <input type="checkbox"/> Power training    |
| <input type="checkbox"/> Circuits          | <input type="checkbox"/> Circuits          |

How many times per week do you/did you take part in resistance training?

... ..

**These questions relate to your main sport/activity**

What is your main sport?

...

<i>On average over the last year:</i>	<i>During a typical year when you were most highly trained</i>
---------------------------------------	--



How often do you/did you train per week?	...	...
How long does/did one training session last?	(minutes)	(minutes)
What is your typical training load in hours or km's per week?	(km/week)	(km/week)
	(hrs/week)	(hrs/week)
How often do you/did you push yourself to your physical limits during training?	<input type="checkbox"/> Never	<input type="checkbox"/> Never
	<input type="checkbox"/> Rarely	<input type="checkbox"/> Rarely
	<input type="checkbox"/> Occasionally	<input type="checkbox"/> Occasionally
	<input type="checkbox"/> Once a week	<input type="checkbox"/> Once a week
	<input type="checkbox"/> Several times/week	<input type="checkbox"/> Several times/week

These questions just relate to cycling. Ignore if you are not a regular cyclist.

	<b>CURRENT</b>	<b>FORMER</b>
What kind of cycling do you currently/did you previously take part in most regularly?	<input type="checkbox"/> Mountain biking	<input type="checkbox"/> Mountain biking
	<input type="checkbox"/> Road cycling	<input type="checkbox"/> Road cycling
	<input type="checkbox"/> Cycle Touring	<input type="checkbox"/> Cycle Touring
	<input type="checkbox"/> Cyclo-Cross	<input type="checkbox"/> Cyclo-Cross
	<input type="checkbox"/> Triathlon/Duathlon	<input type="checkbox"/> Triathlon/Duathlon
	<input type="checkbox"/> Time Trials	<input type="checkbox"/> Time Trials
	<input type="checkbox"/> Other (please provide details)	<input type="checkbox"/> Other (please provide details)
When did you start training regularly for cycling?	(Year)	
Are you/were you actively participating in races?	<input type="checkbox"/> Yes	<input type="checkbox"/> Yes
	<input type="checkbox"/> No	<input type="checkbox"/> No

If so, what kind of races do you/did you primarily take part in? (tick all that apply)

- |   |          |   |          |
|---|----------|---|----------|
| <input type="checkbox"/> XC Biking                                    | Mountain | <input type="checkbox"/> XC Biking                                    | Mountain |
| <input type="checkbox"/> Downhill MTBiking                            |          | <input type="checkbox"/> Downhill MTBiking                            |          |
| <input type="checkbox"/> Road Racing                                  |          | <input type="checkbox"/> Road Racing                                  |          |
| <input type="checkbox"/> Cyclo-Cross                                  |          | <input type="checkbox"/> Cyclo-Cross                                  |          |
| <input type="checkbox"/> Triathlon/Duathlon (Sprint/Olympic Distance) |          | <input type="checkbox"/> Triathlon/Duathlon (Sprint/Olympic Distance) |          |
| <input type="checkbox"/> Half Ironman/ Ironman                        |          | <input type="checkbox"/> Half Ironman/ Ironman                        |          |
| <input type="checkbox"/> Time Trialling                               |          | <input type="checkbox"/> Time Trialling                               |          |
| <input type="checkbox"/> Other (please provide details)               |          | <input type="checkbox"/> Other (please provide details)               |          |

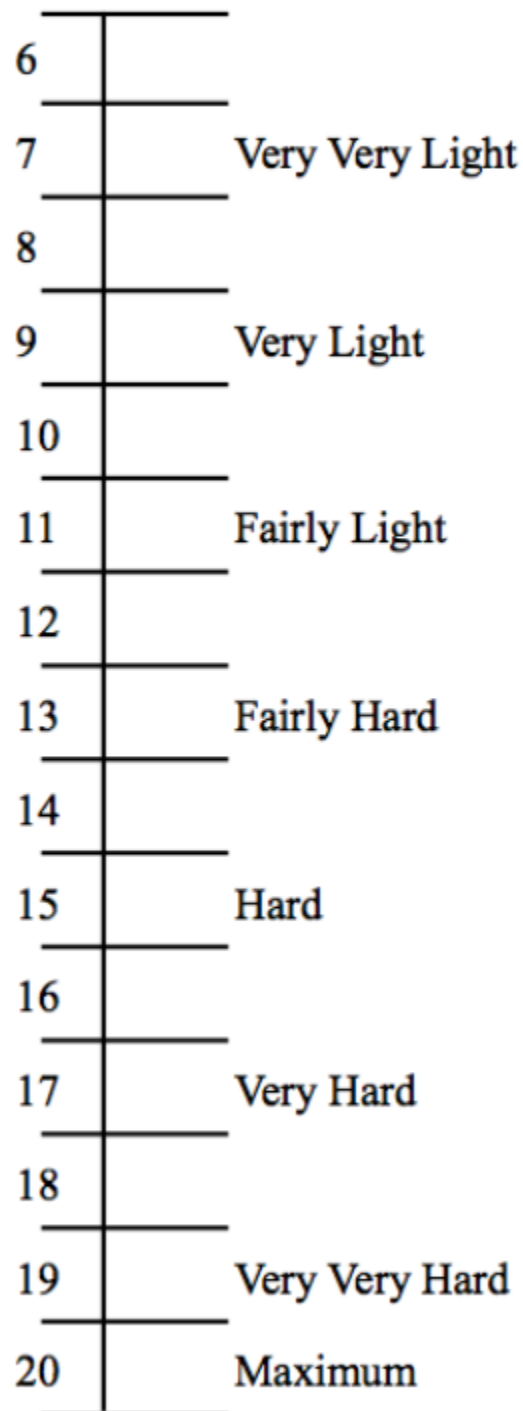
Do you currently/did you previously hold a British Cycling Racing License?

- |   |          |   |          |
|---|----------|---|----------|
| <input type="checkbox"/> Yes 1/Elite                        | Category | <input type="checkbox"/> Yes 1/Elite                        | Category |
| <input type="checkbox"/> Yes Category 2                     |          | <input type="checkbox"/> Yes Category 2                     |          |
| <input type="checkbox"/> Yes Category 3                     |          | <input type="checkbox"/> Yes Category 3                     |          |
| <input type="checkbox"/> Yes Category 4                     |          | <input type="checkbox"/> Yes Category 4                     |          |
| <input type="checkbox"/> Yes Other (please provide details) |          | <input type="checkbox"/> Yes Other (please provide details) |          |
| <input type="checkbox"/> No                                 |          | <input type="checkbox"/> No                                 |          |

If you have raced in the last 5 years what is your best competitive result? ....

Appendix 2

### Rating of Perceived Exertion

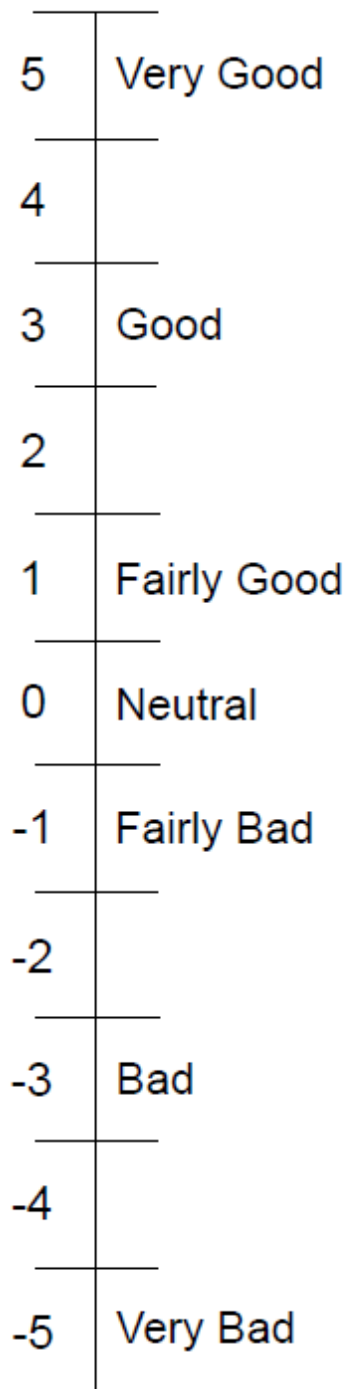


*Please rate your level of exertion at this particular moment*

### Appendix 3

## Feeling Scale

Please rate how you **feel** at this particular moment

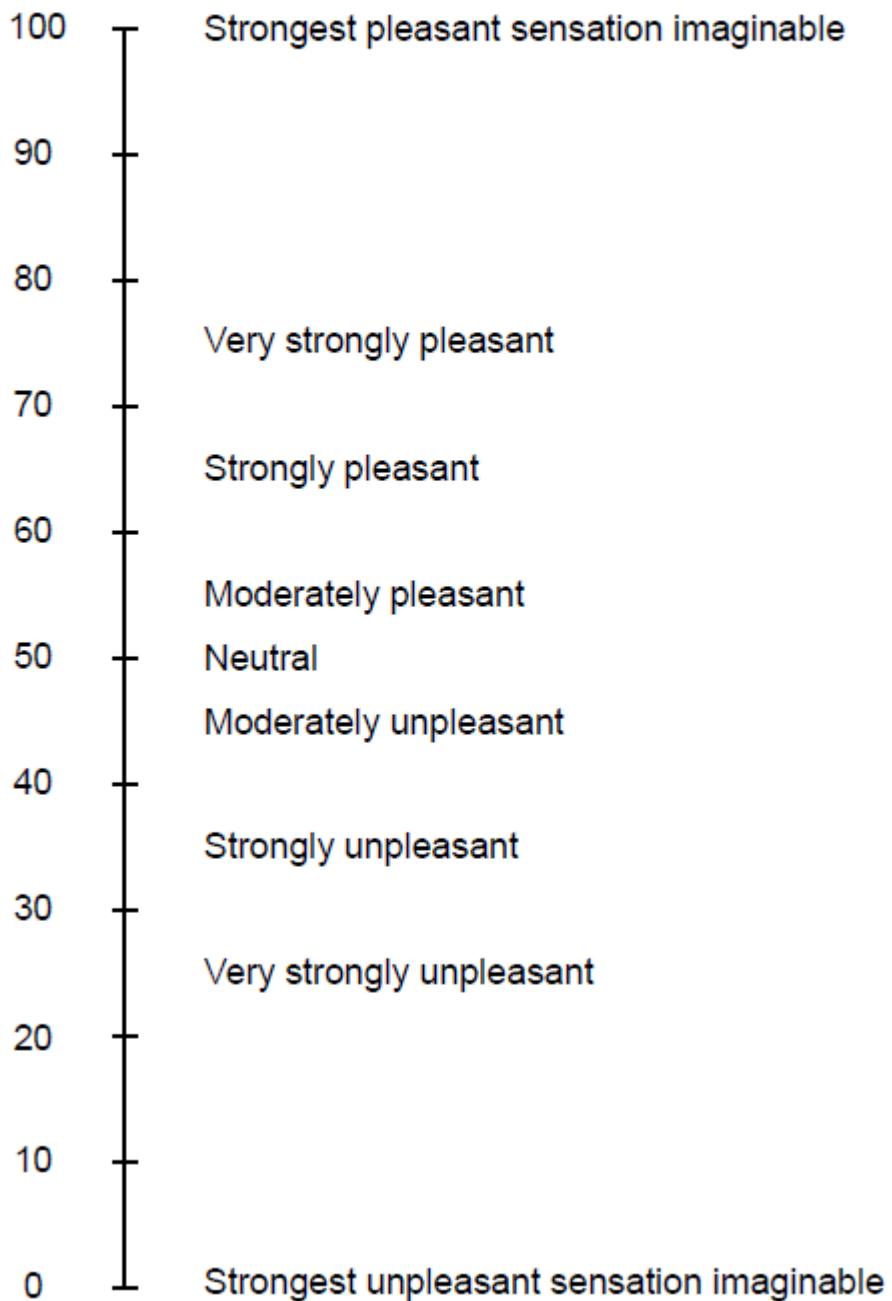


*While participating in exercise, it is quite common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasurable. Additionally, feelings may fluctuate across time. That is, one might feel good and bad a number of times during exercise. This scale measures such responses.*

Appendix 4

## Drink Pleasantness Rating Scale

*Please rate how pleasant you find the drink at this particular moment*



## Appendix 5

### Gastrointestinal Symptoms Questionnaire

*Please select the number on the scale (for each item) that represents the severity of your gastrointestinal symptoms at this particular moment in time*

#### Upper abdominal problems

	None		Mild		Moderate		Severe		Unbearable
	0	1	2	3	4	5	6	7	8
Heartburn									
Bloating									
Nausea									
Vomiting									

#### Lower abdominal problems

	None		Mild		Moderate		Severe		Unbearable
	0	1	2	3	4	5	6	7	8
Intestinal cramps									
Abdominal pain									
Flatulence									
Diarrhea									

#### Systemic problems

	None		Mild		Moderate		Severe		Unbearable
	0	1	2	3	4	5	6	7	8
Dizziness									
Headache									
Muscle cramp									
Urge to urinate									

## Appendix 6

### Exit Questionnaire

1. On which of the two trials do you think you were given the experimental drink?

A. Trial 1

B. Trial 2

2. Did you think your time trial performance was different between the two trials?

A. Trial 1 was a lot better

B. Trial 1 was a little better

C. Both trials were about the same

D. Trial 2 was a little better

E. Trial 2 was a lot better

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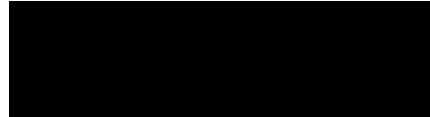
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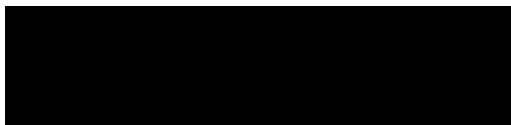


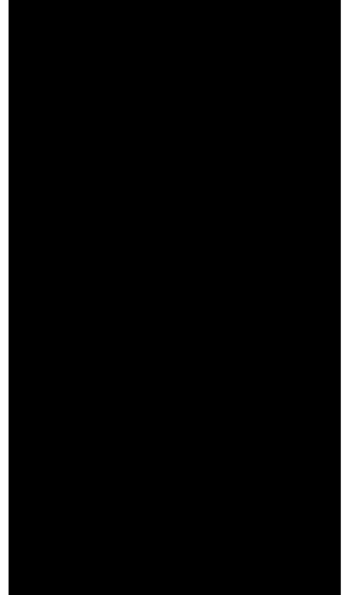
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Telephone: 01865 282246  
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London  
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Invoice Number	13/0001
Date of invoice	24/05/2013

Initial Costs, as agreed	£35095.50
VAT @20%	£7019.10
Total	£42114.60

Terms: 28 days  
Please submit payment, by BACS to:

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[Redacted]

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Professor Kieran Clarke  
Department of Physiology, Anatomy and Genetics,  
Sherrington Building  
University of Oxford  
Oxford,  
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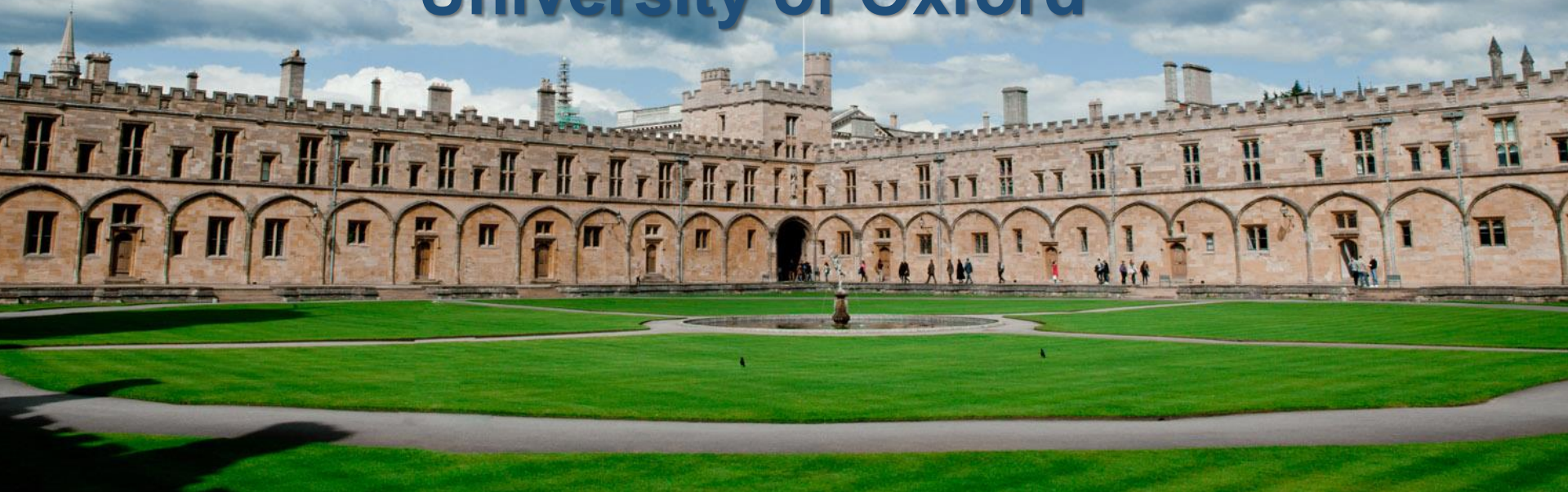
**A new food group to enhance physical performance and manage common diseases**



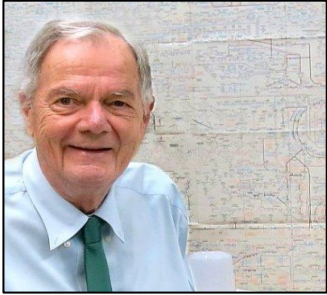
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# **Professor Kieran Clarke**

## **University of Oxford**



# 1993: Insulin or ketones increase cardiac efficiency



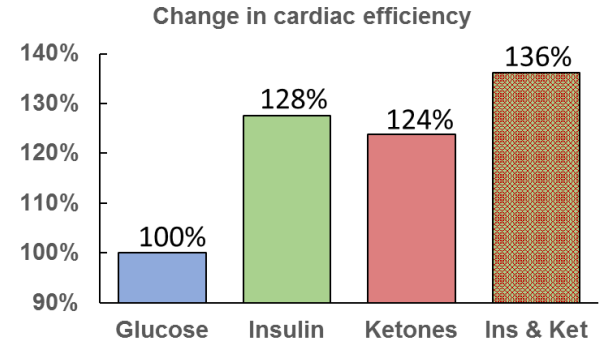
Dr Richard Veech

National Institutes of Health, US



Dr Claudia Keon

University of Oxford





**2003**

*Defense Advanced Research Projects Agency (DARPA)*

Dr Joe Bielitzki  
Programme Manager  
DARPA 2000-2005

## **Peak Warfighter Performance through Mild Ketosis**

Kieran Clarke, Oxford

Richard Veech, NIH

\$9.9 million







# 2003-2005: Ketone ester

Drs Jeremy Robertson and Kirill Tchabanenko  
Chemistry, University of Oxford



Dr Richard Veech and Todd King  
National Institutes of Health, US



Defense Advanced Research Projects Agency (DARPA)  
Peak Warfighter Performance through Mild Ketosis



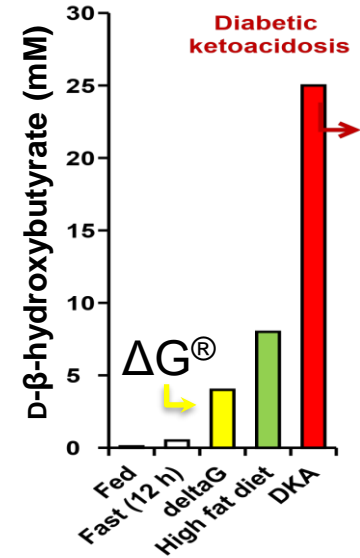
# (R)-3-Hydroxybutyl (R)-3-Hydroxybutyrate



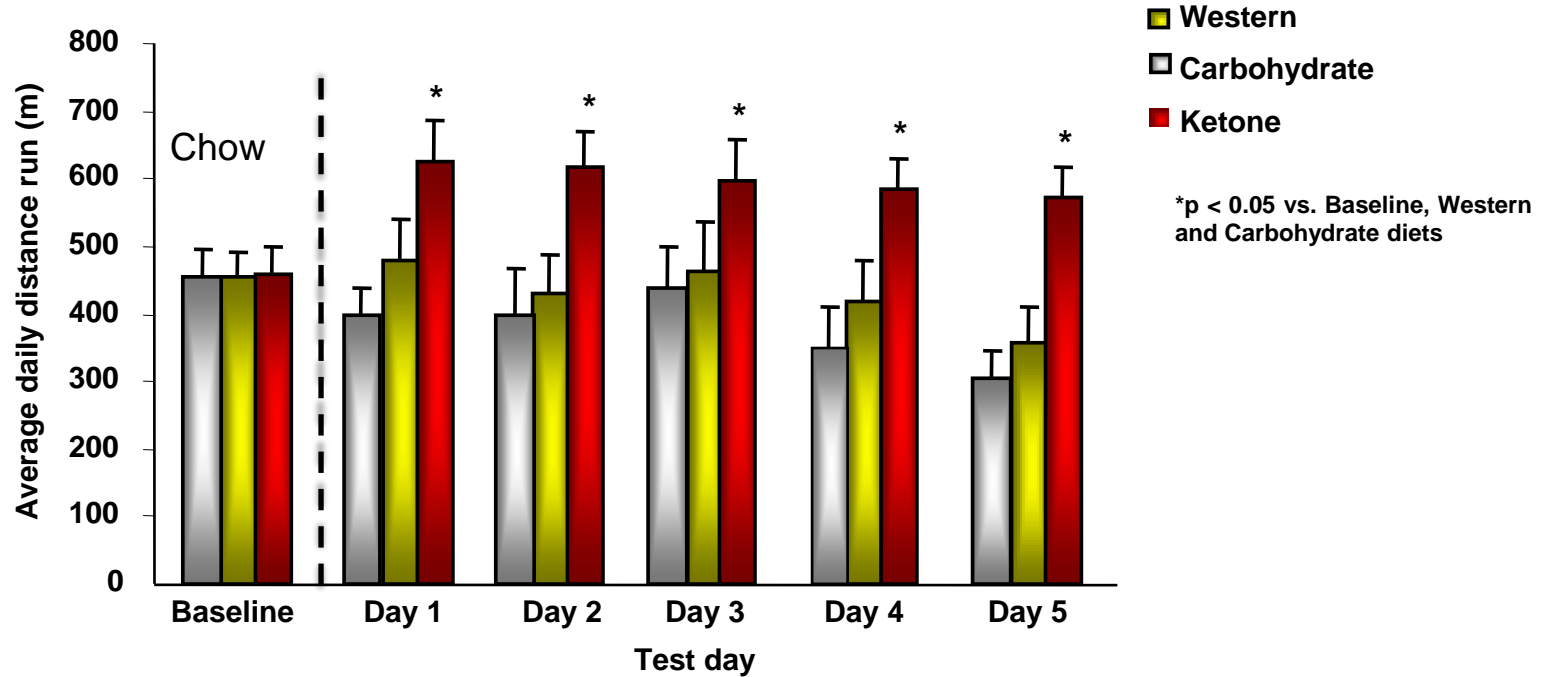
Gibbs-Helmholtz Equation:  $\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$



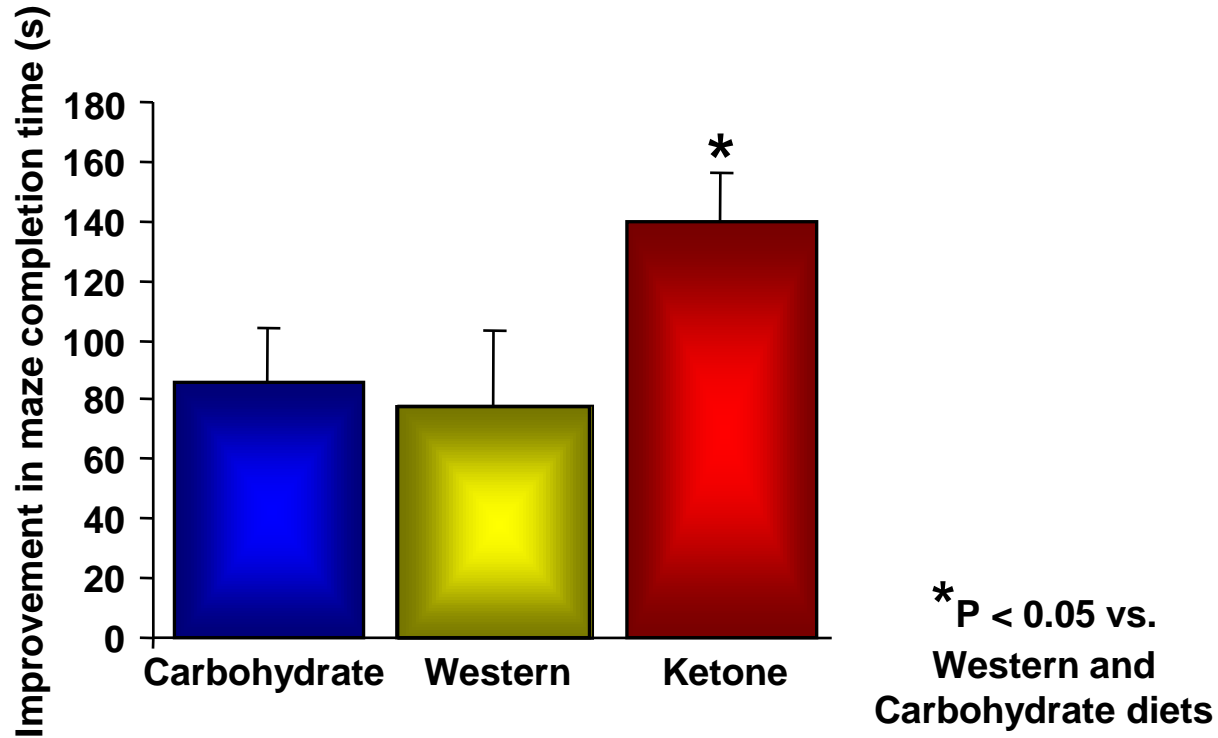
Normal vs. abnormal ketone levels



# $\Delta G^{\circ}$ Enhanced physical performance in rats



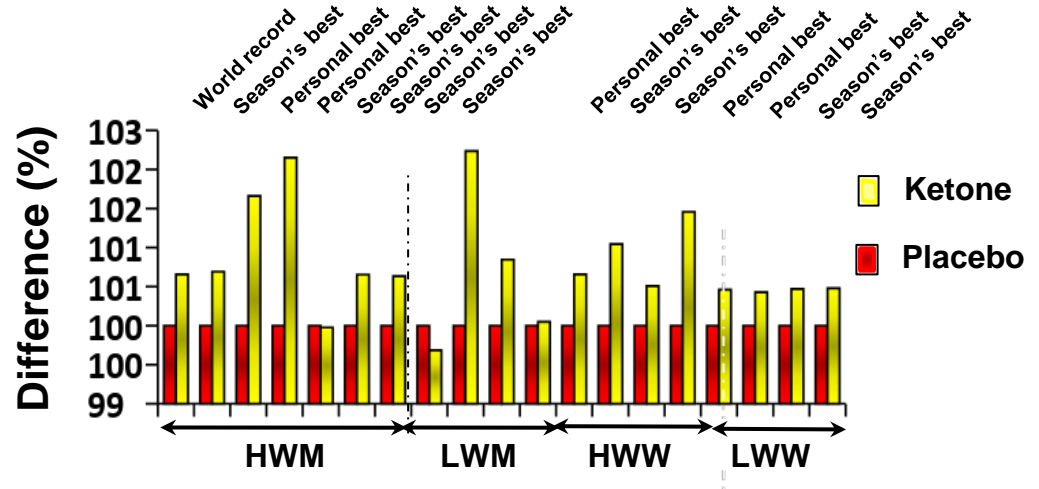
# $\Delta G^{\text{®}}$ Enhanced cognitive function in rats





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