A comparison of fuel use between cycling and running following the co-ingestion of glucose and fructose.

Project Summary:
At present we do not know the effects of the co-ingestion of glucose and fructose on the enzymes regulating substrate oxidation during cycling and running, therefore there is a need to establish the response of these regulatory enzymes with the co-ingestion of glucose and fructose. The aim of this study is to determine the effects of the co-ingestion of glucose and fructose on substrate oxidation and the enzymes responsible for regulating this in endurance cycling and running at high intensity. In a single blind randomised crossover design with 4 conditions, participants will perform experimental trials of steady state exercise at 77%VO_{2max} followed by either a 6 km running time-trial or a 16 km cycling time-trial with and without the ingestion of a ^{13}C labelled 11.25% carbohydrate beverage. Samples of expired air will be analysed for the $^{12}C/^{13}C$ ratio to determine the exogenous oxidation of the ingested beverage as well as the endogenous oxidation of muscle and liver glycogen. Blood samples will be analysed for glucose, lactate, insulin, and free fatty acids. Muscle biopsies will be taken to allow the further quantification of substrate oxidation and identify changes in the phosphorylation state of the relative regulatory enzymes within and between the different experimental conditions. Muscle samples will be analysed for fibre type, glycogen concentration, intramyocellular triglycerides (IMTGs), glycerol content and the enzymes responsible for substrate regulation, including glycogen synthase, glycogen phosphorylase, Hormone Sensitive Lipase (HSL), and Adipose Triglyceride Lipase (ATGL).

Overview:
The ergogenic benefits of carbohydrate ingestion in low to moderate intensity (30 – 75% VO_{2max}) endurance performance (Coggan & Coyle, 1991) and in higher intensity (>75% VO_{2max}) time trial and time to exhaustion performance have been well reported (Below et al., 1995; el-Sayed et al., 1997; Jeukendrup, 2004). Much of this research has used glucose as a sole source of carbohydrate, however, co-ingestion of glucose with a second carbohydrate, such as fructose, has been shown to increase the rates of exogenous carbohydrate oxidation by up to 78 $g \cdot h^{-1}$ (1.3 $g \cdot min^{-1}$) (Jentjens et al., 2004), which may further enhance performance.

Many of these studies use cycling or running as the modes of exercise for their trials, with cycling tending to be the more popular mode (Derman et al., 1996). Indeed, research used to produce guidelines for carbohydrate ingestion during exercise is predominantly based on studies using cycling as the main mode of exercise (Pfeiffer et al., 2011). There are, however, a number of different physiological, metabolic and ergonomic responses to cycling and running (Achten et al., 2003). Despite the importance of these two different modes of exercise, studies to consider the differences in the kinetic response to carbohydrate ingestion during each mode of exercise are limited.

Although an optimal dose of carbohydrate has been identified for cycling time trial performance (Smith et al., 2013), there is no evidence to date suggesting that the same carbohydrate dose will elicit the same percentage improvement in performance amongst runners and cyclists, in a similar time trial scenario. The time trial in this study has been designed to take a similar time period to complete, and will allow a comparison of performance improvement with and without carbohydrate ingestion between the two modes of exercise.

In studies where cycling and running at the same intensity have been compared, running is continually reported to elicit a greater rate of fat oxidation than cycling, even with increased exercise intensity and duration (Achten et al., 2003; Arkinstall et al., 2001; Capostagno & Bosch, 2010; Derman et al., 1996; Pfeiffer et al., 2011; Knechtle et al., 2004). Achten et al. (2003), theorised as the workload in

cycling is distributed over a smaller number of muscle fibres, the muscle strain is more localised, resulting in a higher relative workload on the exercising muscles in cycling compared to running at the same equivalent workload. Subsequently the metabolic stress on each muscle fibre is greater, and the energy requirement of each muscle fibre during cycling is higher. It was hypothesised that this increased energy requirement could only be met by the rapid release of energy from carbohydrate stores. This in turn would cause a higher oxidation of carbohydrate with an associated decrease in fat oxidation (Carter et al., 2000). As we are aware of differences in substrate oxidation between cycling and running, it is important to understand the specific cause of these differences. Muscle biopsies taken during this study will determine the glycogen and IMTG utilisation of the different muscle fibre types during cycling and running.

Carbohydrate ingestion during exercise has been reported to decrease the oxidation of fat (Coyle et al., 1997). As exogenous glucose becomes more readily available at higher ingestion rates, the rate of oxidation increases as oxidation of fat decreases. The elevation of plasma insulin levels as a result of ingesting glucose, has an inhibitory effect on ATGL and HSL which in turn, decreases the breakdown of triglyceride and as a consequence, the concentration of circulating plasma free fatty acids (FFA). Subsequently, there is a decrease in FFA delivery to the muscle during exercise and therefore a decrease in skeletal muscle FFA uptake and oxidation (Coyle et al., 1997; Spriet, 2014). The use of IMTG has been identified as an important substrate in moderate intensity (60% VO_{2max}) activity amongst well trained (VO_{2max} 60.5 ±2.3 ml·kg·min-1) fasted males (van Loon et al., 2003), however, insulin has also been shown to inhibit IMTG lipolysis (Watt et al., 2008). The use of muscle biopsies will allow the quantification of the phosphorylation of these enzymes under the differing metabolic stresses of cycling and running, as well as in the presence and absence of exogenous carbohydrate.

Insulin promotes the uptake of glucose, predominantly into muscle cells, by stimulating the GLUT4 transporter to move in an ATP-independent process from intracellular regions to the plasma membrane. Skeletal muscle accounts for up to ~80% insulin-stimulated glucose uptake in an hyperinsulinemic-euglyceamic clamp (DeFronzo et al., 1985). During exercise, it is believed that skeletal muscle contraction stimulates GLUT-4 exocytosis in an insulin-independent mechanism whilst activation of AMP-dependent Kinase (AMPK) reduces GLUT-4 endocytosis (Richter & Hargreaves, 2013). These events lead to an increased abundance of GLUT-4 at the plasma membrane and a 20 – 30 fold increase in glucose uptake. Muscle biopsies obtained as part of this study will allow the visualisation of GLUT-4 using immunofluorescence, identifying any differences in translocation between different fibre types under the different experimental conditions.

Ingesting carbohydrate during exercise has been demonstrated to maintain euglycemia and attenuate the rates of hepatic glycogenolysis and glucose oxidation (Bosch et al., 1994; Jeukendrup et al., 1999a; Jeukendrup et al., 1999b). Labelling ingested carbohydrate with the stable isotope ^{13}C will allow the quantification of endogenous and exogenous carbohydrate oxidation during cycling and running, combined with the data collected from muscle biopsy samples. This will create a detailed picture of the differences in substrate oxidation between cycling and running with and without the co-ingestion of glucose and fructose.

The purpose of this study is to develop the knowledge and understanding of the co-ingestion of glucose and fructose on substrate oxidation in cycling and running at high intensity and the effects this may have on enzyme regulation and subsequent time trial performance. This study aims to:

- Establish the response of the enzymes regulating substrate oxidation to the co-ingestion of glucose and fructose during high intensity endurance exercise.

- Identify differences in the enzyme response and substrate oxidation to the co-ingestion of glucose and fructose between cycling and running.
- Identify the effects of the co-ingestion of glucose and fructose on time trial performance in cycling and running following a 90 (or120) minute period of high intensity endurance exercise.

Methodology:
Pilot Study; Prior to the commencement of any data collection, 3 participants who satisfy the screening and entry requirements for the study as outlined below will undertake the VO_{2max} running protocol, and subsequent constant intensity running protocol at 77% VO_{2max} to confirm the duration of this component of the study (90 or 120 minutes). During the pilot study, the participants will also consume the carbohydrate beverage that will be provided to participants in the main study to confirm the palatability and volume of the beverage to avoid unnecessary GI discomfort in the main trial. Measures of expired air and heart rate will be taken throughout the VO_{2max} test and constant intensity protocol. Measures of GI discomfort will also be taken at 30 minute intervals during the constant intensity protocol. All participants in the pilot study will attempt to complete 120 minutes of running at 77% VO_{2max}, only if all 3 participants can complete this duration will it be adopted for the experimental trial, otherwise the trial duration will be set at 90 minutes.

Participants:

The study will recruit up to 20 male trained triathletes aged 18 to 35 with a $VO_{2max} \geq 60mL \cdot kg \cdot min^{-1}$ and have a minimum of 2 years triathlon training including at least 3 training sessions per week incorporating at least 1 run and 1 cycle of a minimum duration of 2 hours. It is important that all participants are of a similar training status, as this can have an effects on substrate utilisation (van Loon, 1999). These criteria are based on similar research in this area, that have used participants with a VO_{2max} of ~60 $ml \cdot kg \cdot min^{-1}$ or greater, and the requirement of participants to meet the demands of the high intensity endurance exercise that they will be required to undertake as part of the study. The number of participants is based on the highlighted limitations of similar research that has only identified tendencies in substrate oxidation difference between modes of exercise potentially due to small sample sizes ($n \leq 8$).

All participant will be fit, healthy, non-diabetic and not be currently taking or have recently taken any medications that could interfere with the study. Participants will only be deemed fit for the study following screening, and in accordance with the ACSM risk stratification for atherosclerotic cardiovascular disease, if they are classified as 'low risk'.

Gender; The focus of this study is on male participants, as substrate oxidation differs between genders, and is not an intended focus of this study. Women oxidise a significantly higher percentage of fat than men in both running (~10%) and cycling (~8%) whereas men oxidise greater amounts of carbohydrate (~39 $cal \cdot min^{-1} \cdot kg^{-1}$ in running and~36 $cal \cdot min^{-1} \cdot kg^{-1}$ in cycling) at the same relative exercise intensities (Knechtle et al., 2004; Cheneviere et al., 2011; Dasilva et al., 2011). Dasilva et al. (2011) argued that the magnitude of gender based differences in substrate oxidation increased with greater exercise intensities. Other key research in this area uses only male participants (Pfeiffer et al., 2011; Arkinstall et al., 2001). The effect of hormonal changes amongst female athletes and its effects on substrate oxidisation may warrant further future investigation.

Triathletes; As there are differences in metabolism and the mechanics of running and cycling (Bijker et al., 2002; Achten et al., 2003; Knechtle et al., 2004), the use of triathletes overcomes any complications due to preferential training effects from one form of exercise or the other given that triathletes train for both cycling and running.

Exclusion criteria; Although potential participants may meet the training history, gender and age requirements for the study, they must also be screened for history of illness and disease as well as signs and symptoms of disease, those participants identified as moderate or high risk according to the guidelines of the ACSM (2013) will be excluded from testing. Individuals suffering from Diabetes Mellitus will be excluded due to the irregular regulation of blood glucose in this population which may have an effect on the outcomes of the study. Those participants indicating a food intolerance or allergy to fruit would also be excluded due to the use of fructose in the experimental beverage. Individuals who do not absorb food normally or have bloating side effects would be excluded as failure to absorb the glucose and fructose beverage fully or the bloating side effects caused by an allergy or intolerance could lead to inaccurate measures of substrate oxidation and unreliable reporting of GI discomfort during the trials amongst these individuals. Individuals with blood disorders, including anaemia, haemophilia, HIV, Hepatitis B and C, would also be excluded for the safety of themselves and those taking blood samples. Following completion of the preliminary run test to determine VO_{2max}, any participant failing to achieve a $VO_{2max} \geq 60mL \cdot kg \cdot min^{-1}$ will be excluded from any further participation in the study.

Study design; The aim of this study is to determine the effects of co-ingesting glucose and fructose on substrate oxidation in endurance cycling and running at high intensity. Participants will blindly be provided with a beverage, either a flavoured control (0% carbohydrate) or an 11.25% carbohydrate beverage containing glucose and fructose in a 2:1 ratio. Substrate oxidation will be assessed using the stable isotope tracer ^{13}C, and indirect calorimetry during 90 or 120 minutes of constant intensity treadmill running or ergometer cycling at 77% VO_{2max}. Samples of blood will be collected every 15 minutes throughout the constant intensity exercise, and analysed for glucose, plasma glucose, insulin, free fatty acids and lactate. Muscle tissue samples will be collected pre and post-exercise, and analysed for muscle fibre type, glycogen, lipid content, enzyme activation and the protein content of lactate, fatty acid and glucose transporters by histology and western blotting. Following the period of constant intensity exercise, participants will complete a performance time-trial, running 6 km or cycling 16 km as quickly as possible. In a repeated measures design, all participants will undertake both cycling and running trials with both the experimental carbohydrate beverage and the control 0% carbohydrate beverage. Participants will be asked to attend the laboratory on 6 separate occasions as outlined below;

1. Cycle VO_{2max} baseline test
2. Running VO_{2max} baseline test
3. Experimental trial running with carbohydrate beverage*
4. Experimental trial cycling with carbohydrate beverage*
5. Experimental trial running with 0% carbohydrate control beverage*
6. Experimental trial cycling with 0% carbohydrate beverage*

*The order of trials 3-6 above will be randomly assigned and therefore may vary between individual participants.

Screening: Prior to any testing, all participants will complete a screening questionnaire (appendix A) in line with the guidelines of the ACSM (2013) and will only be considered for the study if they are categorised as 'low risk'. Each participant will also receive a participant information sheet (appendix B). Individuals indicating a positive response to blood disorders (Q29 of the screening from, appendix A) will be excluded from the study even if all other responses categorise the participant as 'low risk'.

Pre-test instructions: Prior to the preliminary bike and run tests for $VO_{2max,}$ participants will be instructed to keep a 3 day weighed food record diary (appendix C) which will be analysed using

Nutritics dietary analysis software to allow the development of a standardised food package for the 24 hour period prior to each experimental trial based on the macronutrient content of the habitual diet, and to allow repetition of the diet in the three days prior to this. Participants will be advised of the requirement to avoid alcohol and high intensity exercise and physical activity for 48h prior to the test. Participants will be instructed not to consume foods with a high natural abundance of ^{13}C, including carbohydrate from C4 plants such as maize and sugar cane for 7 days prior to each trial (Appendix G). This process in combination with their regular training regimen will reduce any background ^{13}C, ensuring that any ^{13}C detected during the experimental protocols is from the labelled carbohydrate ingested as part of the experimental procedure (Jeacocke & Burke, 2010). For the 24 hour period preceding each experimental trial participants will be provided with an individually tailored food package based on the macronutrient content of their habitual diet as recorded in the 3 day food diet, to eliminate variability between trials (Jeacocke & Burke, 2010). Participants will tick off items on a checklist when consumed in addition to noting any additional foods consumed in this period. Participants will be instructed to consume 500mL of water in the 2 hours before going to bed on the night prior to the test to try and ensure euhydration on the morning of the test (Godek et al., 2005), which will be checked on arrival by urine specific gravity, to be ≥1.025. Participants will use the food record diary to replicate their diet prior to each test. Participants must fast for 10 hours prior to any testing procedures, although water may be consumed in this period including the morning of the test.

Preliminary bike test to determine VO_{2max}; Participants will undertake this test at least one week prior to the first experimental trial. Having not conducted intense physical activity for 48 hours prior to the test, participants will arrive following the pre-test instructions outlined previously to them. Upon arrival, participants will void their bladder and have their body mass recorded. Protocols will be based on the research of Coyle et al. (1988), undertaking tests of 2 minute stages lasting between 8 and 10 minutes. A modified version of the protocol used by Robergs et al. (1991) will be used. The test will commence with cycling for 2 min at 100 W, followed by incremental steps of 50 W every 2 min until an intensity of 200 W is reached, thereafter every 2 minutes intensity will increase by 25 W every 2 minutes until volitional exhaustion. Heart rate and samples of expired air will be collected continuously throughout the test to determine exercise intensity and the volume of oxygen utilised at each intensity (Achten et al., 2002; Achten et al., 2003; Pfeiffer et al., 2011). The VO_{2max} value achieved during this test will be used to determine the Wattage required to achieve 77% VO_{2max} for the subsequent constant intensity component of the cycling experimental trials.

Preliminary run test to determine VO_{2max}; Separated by a minimum of 7 days from the preliminary bike test, and following the same pre-test instructions, participants will undertake a treadmill based incremental maximal test to determine running VO_{2max}. As with the preliminary cycle test, upon arrival, participants will void their bladder and have their body mass recorded. To ensure parity between the cycle and run test, the principals of Coyle et al. (1988) will again be employed, and participants will follow the protocol of Costill and Fox (1969) running at a constant speed of 14 $km \cdot h^{-1}$ at 0% gradient for the first 2 minutes, thereafter the gradient will increase by 2% every 2 minutes until volitional exhaustion. Heart rate and breath by breath gas samples will be collected continuously throughout the test (Achten et al., 2002; Achten et al., 2003; Pfeiffer et al., 2011). The VO_{2max} value achieved during this test will be used to determine the suitability of the participant to continue with the experimental protocol (VO_{2max} ≥60 $ml \cdot kg \cdot min^{-1}$) and the relative running speed required to achieve 77% VO_{2max} for the subsequent constant intensity component of the running experimental trials. Each increase in gradient will be converted to running speed using the equations of Margaria et al. (1963), where a 1.5% rise in gradient equals an increase in running speed of 1 $km \cdot h^{-1}$. Participants who fail to achieve a VO_{2max} ≥60 $ml \cdot kg \cdot min^{-1}$ during this test will at this point be exclude from the study.

Experimental Trials; Participants will arrive at the laboratory between 06:00 and 09:00 following a 10h overnight fast. Upon arrival participants will void their bladder and have their body mass recorded. Participants will be allowed to rest for 30 min in a supine position during which time a cannula will be inserted into an antecubital vein, by a researcher trained in phlebotomy using standard sterile techniques. A 10 mL sample of venous blood will be collected for determination of resting plasma metabolite concentrations including glucose, free fatty acids, glycerol, insulin, lactate, plasma glucose, and for $^{13}C/^{12}C$ also. A 10 mL sample of expired air will also be collected for baseline measurement of $^{13}C/^{12}C$ ratio. After 30 min of supine rest and prior to each muscle biopsy, the skin surrounding the quadriceps will be cleaned with Betadine solution. Approximately 60 seconds after cleaning the skin, a local anaesthetic (lignocaine) will be administered to the skin and the deeper tissue and muscle quadriceps fascia. A total of 5 ml anaesthetic will be used. A small incision will be made in the lateral portion of the m. vastus lateralis approximately 25–50% of the distance from the lateral joint line and the greater trochanter. Tissue will be withdrawn (~100 mg) using Bergstrom biopsy needles. Muscle biopsies are commonly practiced in our laboratory and will be taken by clinically trained and/or accredited members of staff. Steristrips will then cover the incision and a dressing will be firmly placed over the steristrips, an ice pack and firm pressure will be applied over the incision to reduce the risk of bleeding, bruising and inflammation. A 10 to 30 min recovery period is offered (or more if needed) before beginning the acute exercise bout. Muscle biopsies will allow the investigation of the acute effect of cycling or running at high intensity with and without the ingestion of carbohydrate on skeletal muscle. The biopsies collected immediately after an acute bout of 90 or 120 min of cycling and running exercise at 77% VO_{2max} will allow the investigation of the acute responses of the skeletal muscle to high intensity endurance exercise with and without carbohydrate ingestion. Immunoflourescence and western blot analyses will be conducted on the muscle samples to investigate the acute molecular responses to exercise Muscle tissue samples will be analysed for fibre type, glycogen, intramyocellular triglycerides, glycerol content and the enzymes responsible for substrate regulation, including glycogen synthase, glycogen phosphorylase, HSL, and ATGL. Participants will cycle or run at 77% of VO_{2max} for 90 or 120 min to investigate the effects of carbohydrate ingestion on substrate oxidation. Over the duration of the constant intensity work period, participants will ingest 1200 mL (90 minutes) or 1600 mL (120 minutes) (800 $mL \cdot h^{-1}$) of either an 11.25% 2:1 glucose:fructose carbohydrate beverage, labelled with 225 ml (90 minutes) or 300 ml (120 minutes) of a ^{13}C tracer to determine the ratio of exogenous to endogenous carbohydrate oxidation, or flavoured, 0% carbohydrate, water (control). Feedings will be given as a 300 mL bolus upon the commencement of exercise followed by 150 mL ingestions every 15 minutes throughout the constant intensity exercise period. This will provide participants with a total carbohydrate intake of 135 g or 180 g (90 $g \cdot h^{-1}$, or 1.5 $g \cdot min^{-1}$). Venous blood samples and samples of expired air (10 mL each) will be collected at 15, 30, 45, 60, 75, 90 (105 and 120) minutes of exercise. Expired air will be monitored continuously throughout the test to allow measures of indirect calorimetry for VO_2 and VCO_2 and the calculation of total carbohydrate and fat oxidation. A total of 80 mL (90 minutes) or 100 mL (120 minutes) of blood will be drawn in each experimental trial. A short gastrointestinal tolerance questionnaire (appendix D) will be administered at 30, 60, 90 (and 120) minutes of exercise. Immediately post exercise, participants will return to the bed and undergo a second muscle biopsy according to the procedures mentioned above. Post-exercise biopsies (~100 mg) will be taken from an incision approximately 2 cm proximal to the first incision to minimise the effect of inflammation on muscle cell signalling. Excess blood and visible connective tissue or fat will be removed from the sample before samples will be divided for analysis by histology and western blotting. Samples will be stored at −80 degrees C until analysis is performed. Steristrips and a dressing will be applied to the incisions an ice pack and firm pressure will be applied to limit the risk of bleeding, bruising and inflammation. Participants will also be advised on how to keep the

incisions clean and free from infection. Following 15 min rest (or more if needed) participants will complete either a 16 km cycling time-trial or a 6 km running time-trial in as quick a time as possible.

Ethical considerations:

The main ethical consideration for this study is the use of muscle biopsies, ~100 mg of muscle tissue per biopsy will be withdrawn from the m. vastus lateralis using Bergstrom biopsy needles. Each participant will have 2 muscle biopsy samples taken during each trial, over a total of 4 trials, each participant will have 8 muscle biopsy samples taken equating to ~800 mg of muscle tissue. The size of each biopsy sample and the number of samples taken as part of this study is based on the protocols of other research in this area (Arkinstall et al., 2001) Muscle biopsy samples will allow quantification of fibre type, glycogen, intramyocellular triglycerides, glycerol content and the enzymes responsible for substrate regulation, including glycogen synthase, glycogen phosphorylase, HSL, and ATGL. Following cleaning an application of a local anaesthetic (lignocaine) ~100 mg of muscle tissue per biopsy will be withdrawn from the m. vastus lateralis using Bergstrom biopsy needles. The risks of this procedure involve pain to the participant, which will be minimized by the injection of local anaesthetic and the possible formation of a bruise from localized bleeding. There may be a small amount of pain experienced while the local anaesthetic is administered. Thereafter, the procedure may be associated with a feeling of pressure and/or mild discomfort, but only for a short time. The biopsy procedure has been reviewed by multiple authors (Edwards et al., 1983; Highstead et al., 2005; Tarnopolsky et al., 2011), reporting a 0.38 – 1.10% chance of haematoma, and ≤ 0.13% chance of infection. All biopsy samples will be taken by a licenced health care professional (orthopaedic surgeon or A&E doctor) trained and experienced in the taking of muscle biopsy samples.

Additional ethical considerations relate to maximal exercise testing (VO_{2max}), high intensity exercise, the use of phlebotomy for venous blood sampling and the use of a stable isotope (^{13}C) as a carbohydrate tracer. The selection of participants is appropriate to the design of this study, as they are accustomed to the intensity, mode and duration of activity. Blood sampling will be taken by researchers trained in cannulation and deemed competent by the institutional assessor. As ^{13}C is naturally occurring and present in corn derived food products, its use as a tracer poses little or no risk to participants. Enrichment of the carbohydrate beverages with ^{13}C will be done in accordance with the manufacturers recommendations. A detailed account of the risks to participants and researchers is presented in section 6 'Risk and Benefits'.

Human Participants:

Pilot Study; Prior to the commencement of any data collection, 3 participants who satisfy the screening and entry requirements for the study as outlined below will undertake the VO_{2max} running protocol, and subsequent constant intensity running protocol at 77% VO_{2max} to confirm the duration of this component of the study (90 or 120 minutes). During the pilot study, the participants will also consume the carbohydrate beverage that will be provided to participants in the main study to confirm the palatability and volume of the beverage to avoid unnecessary GI discomfort in the main trial. Measures of expired air and heart rate will be taken throughout the VO_{2max} test and constant intensity protocol. Measures of GI discomfort will also be taken at 30 minute intervals during the constant intensity protocol. All participants in the pilot study will attempt to complete 120 minutes of running at 77% VO_{2max}, only if all 3 participants can complete this duration will it be adopted for the experimental trial, otherwise the trial duration will be set at 90 minutes.

Study; 20 male volunteers aged 18 to 35 will be recruited to participate in the study. All participants will be triathletes with a $VO_{2max} \geq 60mL \cdot kg \cdot min^{-1}$ and have a minimum of 2 years triathlon training

including at least 3 training sessions per week incorporating at least 1 run and 1 cycle of a minimum duration of 2 hours. These criteria are based on similar research in this area, and the requirement of participants to meet the demands of the high intensity endurance exercise that they will be required to undertake as part of the study. The number of participants is based on the highlighted limitations of similar research that has only identified tendencies in substrate oxidation difference between modes of exercise potentially due to small sample sizes (n≤8).

All participants will be fit, healthy, non-diabetic and not be currently taking or have recently taken any medications that could interfere with the study. Participants will only be deemed fit for the study following screening, and in accordance with the ACSM risk stratification for atherosclerotic cardiovascular disease, if they are classified as 'low risk'. Those participants indicating a food intolerance or allergy to fruit would also be excluded due to the use of fructose in the experimental beverage. Individuals who do not absorb food normally or have bloating side effects would be excluded as failure to absorb the glucose and fructose beverage fully or the bloating side effects caused by an allergy or intolerance could lead to inaccurate measures of substrate oxidation and unreliable reporting of GI discomfort during the trials amongst these individuals. Individuals with blood disorders, including anaemia, haemophilia, HIV, Hepatitis B and C, would also be excluded for the safety of themselves and those taking blood samples. Following completion of the preliminary run test to determine VO_{2max}, any participant failing to achieve a $VO_{2max} \geq 60mL \cdot kg \cdot min^{-1}$ will be excluded from any further participation in the study.

Recruitment, Voluntary Participation, Consent and Right to Withdraw:
Participants will be recruited through a combination of posters (appendix E) at local triathlon clubs training venues, and posts on on-line triathlon forums (220 Triathlon, TriTalk.co.uk, Tri247 and Runners World Triathlon Forum).

The research will not be covert in nature, or involve any deliberate deception. It is in the interest of both the researcher and participants to only recruit those individuals who are suitable for the study. All communication with potential participants will be honest, detailed and non-coercive.

All participants will receive an information pack containing a study information sheet, screening form, 3 day diet diary, muscle biopsy information sheet, and a consent form (appendix F) allowing participants to provide informed consent prior to the study. All participants will be informed in the information sheet of their right to withdraw their participation and/or data at any time without giving reason or prior notice, along with details on how to withdraw from the study. All participants completing the trial in full will receive a £50 voucher to compensate them for their considerable time commitment to the study.

Risks and Benefits:
Muscle Biopsies; In total, 8 muscle biopsy samples will be obtained from the lateral portion of the m. vastus lateralis of each participant (4 from each leg). One pre-exercise resting and 1 post-exercise when cycling with carbohydrate ingestion, 1 pre-exercise resting and 1 post-exercise when cycling without carbohydrate ingestion, 1 pre-exercise resting and 1 post-exercise when running with carbohydrate ingestion, 1 pre-exercise resting and 1 post-exercise when running without carbohydrate ingestion. This is equally divided between 4 exercise bouts, trials 1 and 2 separated by 1 week, trials 2 and 3 separated by 2 weeks, and trials 3 and 4 separated by 1 week. Muscle biopsy samples will be taken from alternating legs, a two week recovery period between trials 2 and 3 will

allow recovery of the vastus lateralis muscle used in week 1 prior to its use as a sample site again in week 3.

No research will be conducted for analysis or use of human DNA in the samples.

The risks of this procedure involve pain to the participant, which will be minimized by the injection of local anaesthetic and the possible formation of a bruise from localized bleeding. There may be a small amount of pain experienced while the local anaesthetic is administered. Thereafter, the procedure may be associated with a feeling of pressure and/or mild discomfort, but only for a short time. Bleeding from the biopsy site is unlikely since no major artery or vein is located in the area of the m. vastus lateralis. The only possible complication of this procedure is infection due to lack of sterility during the procedure. We will minimize the risk of infection by using strict sterile protocols during all invasive procedures. Risks to the participant can include bruising, infection or insensitivity of the skin although these risks are very small. Risks to the qualified personnel conducting the biopsies include the direct skin contact with the participant's blood and tissue. Standard clinical hygiene and sterile protocols will minimize such risks.

The biopsy procedure has been reviewed by Edwards et al (1983). The research group reported that from 800 biopsies taken in their laboratory only 3 cases of haematoma occurred and just 1 case of infection, which was a minor skin infection and the group reported no cases of deep muscle infection or insensitivity of the skin. The procedure was further reviewed by Highstead et al (2005). They reported that from 1,133 biopsies performed on volunteers aged below 60 years, there was a very small chance of haematoma (1.1%) and no incidence of infection. Finally, in a study of over 13,500 muscle biopsies performed by Tarnopolsky et al (2011) in adult lean and obese research subjects aged 18 – 89 years there was more than 99% success rate and a minor complication rate of 0.15%. The chance of haematoma will be further minimized by applying an ice pack with a compression bandage to provide firm pressure over the biopsy site (in young individuals this is approximately 3 min).

Participants will be monitored by a member of the research team (for more than 1 hour if required) after the biopsy and sent home with prearranged transport by car or taxi. They will be given a telephone number of a member of the research team who will be on call for the rest of the day in the unlikely case that an emergency would occur despite the extra precautions.

All muscle biopsy samples will be taken by a licenced orthopaedic surgeon or A&E doctor, and will be stored at St James's University Hospital, Leeds under their human tissue act licence (Appendix H). In accordance with this procedure a copy of the participants consent form must be kept with the sample and linked to their identification number. Participants will be informed that their sample may be identifiable by approved researchers under the terms of the human tissue licence act prior to consent being provided.

Exercise; Participants will be required to perform two VO_{2max} tests, 1 on the treadmill and 1 on a cycle ergometer, in addition to performing two high intensity (77% VO_{2max}) running trials and two cycling trials for 90 or 120 minutes followed by a 6 km running time-trial or 16 km cycling time-trial. Maximal exercise testing and high intensity exercise both have inherent risks including fainting, nausea/vomiting, cardiovascular complications and musculoskeletal injuries. All participants will undergo screening to establish medical history and suitability to exercise prior to any testing. Screening will be in accordance with the ACSM risk stratification for cardiovascular disease. Only participants deemed 'low risk' will be permitted to participate in the study. During all trials participants will be continuously monitored during exercise and recovery. Prior to departure from the

laboratory participants will be debriefed and have contact details for the research team should any concerns arise following testing. A researcher trained in first aid will be present throughout the trials.

Cannulation and blood sampling; Repeated venous blood sampling will be undertaken in the experimental trials by the use of a cannula inserted into an appropriate vein of the forearm. Eight (90 min) or ten (120 min) venous blood samples (10 mL each) will be collected from each volunteer during each experimental trial (32 or 40 venous samples in total, 320 or 400 mL blood collected). All researchers carrying out phlebotomy will be trained and deemed competent by the institutional phlebotomy trainer and assessor. All blood sampling will be carried out safely using appropriate procedures, by trained researchers wearing appropriate personal protective equipment. Aseptic methods of sampling will be used to minimise the risk of infection and disposed of in a safe manner following approved laboratory procedures. Blood samples will be securely stored in a freezer in the laboratory of the CRI at Leeds Beckett University and following analysis will be disposed of following appropriate laboratory procedures in accordance with the health and safety guidelines (segregation of sharps and soft waste into appropriate clinical waste systems). The procedures for the storage of blood plasma and serum do not come under the conditions governed by the Human Tissue Act.

Beverage Ingestion; The study will use two beverages both made by the researchers, one 11.25% 2:1 glucose: fructose carbohydrate beverage, labelled with a ^{13}C tracer, and one flavoured, 0% carbohydrate, water (control). ^{13}C is a stable isotope which does not decay over time or emit radiation (Mahon & Timmons, 2014). Stable isotopes are commonly used as tracers in studies of exercise metabolism as they are metabolically similar to the unlabelled compound being studied (Timmons et al., 2003). The side effects associated with the use of ^{13}C are minimal or non-existent (Bodamer & Halliday, 2001; Jones & Leatherdale, 1991; Koletzko et al., 1997), as sugars enriched with ^{13}C are naturally occurring, produced by plants using the C4 chain of photosynthesis, e.g. corn (Mahon & Timmons, 2014). Participants may have allergies of hypersensitivity to the ingredients used in the development of these beverages, drinks also present a risk of contamination. All beverages will be prepared by a researcher using appropriate food grade ingredients and measures from approved suppliers. Products will be stored appropriately to prevent spoilage and contamination. All participants will be made aware of the type of beverages and their ingredients as part of the information provided to them in the screening and recruitment process. Any allergies and intolerances will be discussed and individuals presenting allergies or intolerances to any of the ingredients will be removed from the study.

Risks to the researcher; All risks will be managed by ensuring that all researchers are trained and competent in the correct use of equipment and procedures. Specific risks relate to the use of blood sampling and the use of liquid nitrogen for the freezing of collected muscle biopsy samples.

Blood sampling will require the wearing of personal protective equipment at all times, including laboratory coats and disposable gloves. Sampling will use sterile equipment disposed of following the specified institution procedures.

Liquid nitrogen will be held in a vented dewar and stored in a cage with appropriate warnings. The room where the liquid nitrogen is stored has sufficient ventilation (12 air changes per hour) to prevent a dangerous decline in O_2 levels and if fitted with an alarmed O_2 sensor (with remote warning alarms at all entrance and exit points to the storage room) to alert researchers and other laboratory users to the danger of asphyxiation. If all of the contents of the full 25 litre dewar were to turn immediately to nitrogen gas, the resultant O_2% (~16%) would be similar to that experienced in the university's environmental chamber which has been tolerated for prolonged periods of time, however under these circumstances entry to the room would still be prohibited until such time that the O_2% had returned

to a safe level (≥20%). A small amount of liquid nitrogen (< 1 litre) will be decanted into an open dewar during each trial. The main storage dewar will be fitted with a dispenser, allowing for safe filling. The liquid nitrogen will be handled with the appropriate safety equipment (cryogenic gloves, face mask and laboratory coat) to prevent cryogenic burns or frostbite. All procedures have been approved by health and safety to ensure that they satisfy current regulations. Researchers have undertaken training relevant to the handling of liquid nitrogen.

Personal Data, Anonymity and Confidentiality:
Each participant will be given a unique code for the duration of the study, a record of which will be retained by the lead researcher. Upon completion of data analysis and feedback to participants where requested, this file will be deleted and hard copies disposed of in confidential waste. Where data may be published, mean group values will be reported, if however it is deemed appropriate to some individual results should be reported to support the interpretation of data, again the non-identifiable number will be used. All stored blood samples will be stored to maintain anonymity. Although muscle biopsy samples will be coded, in accordance with the Human Tissue Act Licence, a signed copy of the participants consent form must be kept with the sample, making each sample identifiable by a limited number of individuals in addition to the lead researcher for record keeping purposes only. This information will be conveyed to all participants, and they will consent to allowing this on the informed consent form.

The data and information will be classified as new data and is currently not in the public domain. Personal data will be coded at the start of the study by the principal investigator who with the exception of the muscle biopsy samples identified above, will be the only person able to trace data back to any participant. The principal investigator will store this coded information on a password protected computer. Data will not be presented at any point with real names. The data from the study will be kept for 10 years following the completion of the study, thereafter it will be destroyed.

Reporting and Dissemination:
This study will contribute towards my doctoral thesis, and is intended for publication at conferences or in peer reviewed journals. No additional permissions will be required for this. Undergraduate and postgraduate students assisting in the data collection process will also have access to anonymised data for the completion of their own major independent studies.

Appendix A

School of Sport – Physiology Group Screening Questionnaire

Name:_____

Student ID:_____

Risk Factors		**Risk Factor**	**No Risk Factor**
Q 1. Age = _____ years	Males	**≥ 45**	**< 45**
	Females	**≥ 55**	**< 55**
Q 2. Have any parents, brothers or sisters had a heart attack, bypass surgery, angioplasty, or **sudden death*** prior to 55 years (male relatives) or 65 years (female relatives)		**Yes**	**No**
Q 3. Are you currently a smoker – have you quit within the past 6 months – are you exposed to environmental tobacco smoke?		**Yes**	**No**
Q 4. In the past 3 months have you performed at least 30 minutes of moderate intensity physical activity or equivalent on at least 3 days of the week (total of 90min mod. act.)?		**No****	**Yes**
Q 5a. Body mass index = _____ $kg.m^{-2}$ (weight divided by height squared)		**≥ 30**	**< 30**
Q 5b. Waist girth = _____ cm	Males	**> 102**	**≤ 102**
	Females	**> 88**	**≤ 88**
Q 6a. Do you take blood pressure medication		**Yes**	**No**
Q 6b. Resting blood pressure: SBP = _____ *mmHg, DBP* = _____ *mmHg*		**≥ 140/90*** **	**< 140/90**
TOTAL NUMBER OF RISK FACTORS * If Yes to **<u>early</u> sudden death** in family history advise pre-participation screening for SCD ** If currently sedentary consider whether max test is essential ***If BP ≥140/90mmHg treat as High Risk and advise pre-participation screening for SCD			

Signs or Symptoms	**S/S**	**No S/S**
Q 7. Do you ever have pain or discomfort in your chest or surrounding areas (neck, jaw, arms or other areas)?	**Yes**	**No**
Q 8. Are you ever short of breath at rest or with mild exertion?	**Yes**	**No**
Q 9. Have you ever experienced dizziness or loss of consciousness during or shortly after exercise?	**Yes**	**No**
Q10. Have you ever been short of breath at rest in the recumbent position or had an attack of breathlessness in the middle of the night which was relieved by sitting up?	**Yes**	**No**
Q 11. Do your ankles ever become swollen (other than as a result of an injury)?	**Yes**	**No**

Q 12. Do you ever have palpitations (=the unpleasant awareness of the heart beating in your chest) or an unusual period of rapid heart rate?	**Yes**	**No**
Q 13. Do you ever suffer from cramp-like pains in your legs, brought on by exertion and relieved after 1-2 minutes of rest?	**Yes**	**No**
Q 14. Has a doctor ever said you have a heart murmur?	**Yes**	**No**
Q 15. Do you feel unusually fatigued or find it difficult to breathe with usual activities?	**Yes**	**No**
SIGNS/SYMPTOMS OF DISEASE	**YES / NO**	

Personal History of Disease	**History of Disease**	**No History of Disease**
Q 16. Heart disease	**Yes**	**No**
Q 17. Peripheral vascular disease	**Yes**	**No**
Q 18. Cerebrovascular disease (e.g. stroke)	**Yes**	**No**
Q 19. Chronic obstructive pulmonary disease (emphysema/chronic bronchitis)	**Yes**	**No**
Q 20. Asthma	**Yes**	**No**
Q 21. Interstitial lung disease	**Yes**	**No**
Q 22. Cystic fibrosis	**Yes**	**No**
Q 23. Diabetes mellitus	**Yes**	**No**
Q 24. Thyroid disorder	**Yes**	**No**
Q 25. Renal disease	**Yes**	**No**
Q 26. Liver disease	**Yes**	**No**
HISTORY OF DISEASE	**YES / NO**	

Other conditions	**Condition**	**Condition**
Q 27. Do you have any bone or joint problems such as arthritis or a past injury that might get worse with exercise? **(Exercise testing may need delaying or modifying)**	**Yes**	**No**
Q 28. Do you have any other problem that might make it difficult for you to do strenuous exercise? **Details**:	**Yes**	**No**
Q29. Have you ever been diagnosed with a blood disorder (e.g. anaemia, haemophilia, HIV, Hepatitis B or C)? **Please state:**	**Yes**	**No**
Q30. Are you on any prescription medications? **List:**	**Yes**	**No**

	No RF	**1RF**	**1HoD/SS**
Total cholesterol = _____ mmol.l^{-1}	**< 5.18**	**≥ 5.18**	
***Or** HDL cholesterol* = _____ mmol.l^{-1}*		**< 1.04**	
Fasting glucose = _____ mmol.l^{-1}	**< 5.55**	**≥ 5.55 ≤ 6.94**	**> 6.94**
***Or** Non-Fasting glucose= _____ mmol.l^{-1}*	**< 7.77**	**≥ 7.77 ≤11.04**	**> 11.04**

*If HDL>1.55 – subtract 1 from the total number of risk factors

Risk Analysis

Total no. of RF (inc blood analysis, if necessary) = _____

SS/HoD = YES / NO – Other conditions to consider: YES / NO

Low risk: No more than one risk factor – safe to do submax/max exercise testing or enter a vigorous programme

Moderate risk: More than one risk factors – safe to do submax exercise testing or enter a moderate programme (max test must be conducted by a person trained in clinical exercise testing)

High risk: One or more signs/symptoms of disease or HoD - cannot do any testing or exercise without physician clearance

Final risk category = _____

- I confirm that the above information which I have provided to Leeds Beckett University is true and accurate to the best of my knowledge and belief and I understand that I must notify promptly of any changes to the information.
- I understand that the information I have provided above may be used as part of an anonymised dataset by staff or students of the School of Sport for completion of coursework or for research or audit purposes (with the appropriate ethical approval in place).

Student Signature: **Date:**

Assessors Signature: **Date:**

Appendix B

Participant information sheet

Title of the study:
A comparison of fuel use between cycling and running following the co-ingestion of glucose and fructose.

Introduction:
We are inviting you to take part in a research study. Before you decide whether you would like to participate, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with family and friends if you wish. Please ask us if there is anything that is not clear, or if you would like more information (a contact number and address are at the end of this information sheet).

Background to, and purpose of the study:
The role of ingested carbohydrate during endurance exercise has been greatly researched. Repeatedly improvements in endurance performance have been shown when carbohydrate was ingested during activity compared to water alone.

More recent research has given us an optimal type of carbohydrate, with glucose plus fructose providing the optimal blend for endurance exercise. Recent research has also given us an optimal dose of carbohydrate ingestion to give us the largest increase in performance. Much of this research has been conducted using cycling as the mode of exercise. Other studies have shown that we use differing amounts of fuels (substrates) in cycling and running, with cycling showing greater reliance on muscle glycogen, and running relying more on fat as a source of energy.

Only a very limited number of studies have considered the differences in the substrates used to provide energy in cycling compared to running when carbohydrate is ingested during activity. Although inconclusive, the limited number of studies into this area do suggest that there may be a difference in substrate utilisation between the two modes of exercise.

The current study aims to investigate these differences further and understand the effects that the different modes of exercise have on the enzymes that regulate the substrates used with and without carbohydrate ingestion.

Am I a suitable participant for the study?
We are looking or approximately 20 well trained male triathletes aged 18 to 35 to participate in the study. You must confirm that you are fit and healthy, not diabetic, and not currently taking any medications that may interfere with the study. To be considered a well-trained triathlete, you will have been:

- Triathlon training for a minimum of 2 years
- Completing a minimum of 3 training sessions per week.
- Undertaking least 1 run and 1 cycle lasting a minimum of 2 hours duration per week.

Following screening, you should be classified as “low risk”. You should be free from any form of food intolerance or allergy to fruit. Individuals who do not absorb food normally or have bloating side effects will be excluded.

Individuals with blood disorders, including anaemia, haemophilia, HIV, Hepatitis B and C, will also be excluded for the safety of themselves and those taking blood samples.

Following completion of the preliminary run test to determine maximal oxygen uptake (VO_{2max}), any participant failing to achieve a VO_{2max} of 60mL·kg·min^{-1} or above will be excluded from further participation in the study.

Do I have to take part?
No. It is entirely up to you whether you participate in this study or not. If you do decide to participate, 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 free to withdraw at any point by contacting the lead researcher (details are at the end of this sheet) by phone, e-mail or in person, you do not have to give any reasons or advanced notification.

What will happen if I take part?
Prior to undertaking any form of exercise you will be invited in to the human performance laboratory at Leeds Beckett University to undertake screening to check your suitability for participation in maximal and high intensity exercise. Following satisfactory completion of the screening procedures you will be asked to return to the laboratory on 6 separate occasions: On your first two visits we will measure your VO_{2max} for cycling and running, this will essentially give us your fitness level for each activity, you will then undertake the four experimental trials. The order of the tests is outlined below:

1. Baseline cycling VO_{2max} test
2. Baseline running VO_{2max} test
3. Experimental trial running with carbohydrate beverage*
4. Experimental trial cycling with carbohydrate beverage*
5. Experimental trial running with 0% carbohydrate control beverage*
6. Experimental trial cycling with 0% carbohydrate beverage*

*The order of trials 3-6 above will be randomised, and therefore may not follow exactly the pattern shown above.

Screening:
During your first visit to the laboratory, you will be asked to complete a screening questionnaire, and a researcher will take some baseline measurements of heart rate, blood pressure, waist circumference, height and weight. All participants in the trial are required to be deemed as 'low risk' according to the criteria of the American College of Sports Medicine (ACSM).

If, following the screening, you are deemed to be suitable to participate in the trial and you still wish to do so, you will at this point be provided with a 3 day weighed food diary to complete prior to your first baseline VO_{2max} test. This diary will be used to provide similar eating habits in the lead up to all trials and to inform the development of the 24 hour food package that you will be provided with prior to each experimental trial.

If, following the screening process and analysis of your food diary, you are identified as having a health risk or abnormality you may be withdrawn from the study and asked to consult your GP.

Baseline cycling VO_{2max} test:
We request that you avoid alcohol and high intensity exercise for 48 hours prior to this test.

You will arrive at the laboratory between 06:00 and 09:00 am.

Before commencing exercise we will take another measure of your body mass and ask a few brief screening questions to check that there has been no significant change in your health since you completed the screening questionnaire.

The bike will be set up for you individual requirements, and you will begin a continuous incremental test until voluntary exhaustion.

Throughout the test you will wear a facemask so that expired air can be collected and analysed using a computerised gas analysis system to calculate your VO_{2max}.

The testing procedure will be as follows:

Stage	Duration (min)	Intensity (Watts)
1	2	100
2	2	150
3	2	200
4	2	225
… the 2 minute stages with 25 Watt increases will continue until volitional fatigue		

The research team will monitor your heart rate and expired air throughout the test.

The data collected here will be used to calculate the intensity for the subsequent cycling experimental trials.

Baseline running VO_{2max} test:
This test will take place 7 days after your baseline cycling VO_{2max} test.

Again we request that you avoid alcohol and high intensity exercise for 48 hours prior to the test.

We also request that you follow a similar diet to that which you consumed prior to your cycling VO_{2max} test.

You will arrive at the laboratory between 06:00 and 09:00 am. Before commencing exercise we will take another measure of your body mass and ask a few brief screening questions to check that there has been no significant change in your health since you completed the screening questionnaire.

As with the cycling test, you will wear a facemask so that expired air can be collected and analysed using a computerised gas analysis system to calculate your VO_{2max}.

The testing procedure will be as follows:

Stage	Duration (min)	Speed ($km \cdot h^{-1}$)	Incline (%)
1	2	14	0
2	2	14	2
3	2	14	4
4	2	14	6
… the stages at 14 $km \cdot h^{-1}$ with a 2% increase in incline every 2 min will continue until volitional fatigue			

The research team will monitor your heart rate and expired air throughout the test.

The data collected here will be used to calculate the intensity for the subsequent running experimental trials.

Experimental trials:
7 days after your baseline running VO_{2max} test, you will undertake your first experimental trial. In the days leading up to the trial you will replicate your diet from the food diary as you did for the baseline tests. For the 24 hour period prior to each experimental trial you will be provided will a food package based on the calorie and macronutrient intake from your food diary. You should tick off foods from this package on the list provided as you eat them, and note down any additional items you may consume. You must however fast for 10 hours before each trial, although you are encouraged to still consume water in this period.

The procedures for each experimental trial will follow the pattern described below:

You will arrive at the laboratory between 06:00 and 09:00 am.

Upon arrival the research team will again record your body mass and ask a few screening questions.

You will then rest for 30 minutes, during which time a trained researcher will fit a cannula into a vein of your forearm, to allow small samples of blood to be taken at regular intervals. You will feel a small scratch as the needle enters your vein, but once in place you should not be able to feel the cannula.

A small (10 mL) resting blood sample will be taken and analysed for glucose, free fatty acids, glycerol, insulin and lactate.

Throughout the exercise period, this cannula will be used every 15 minutes to draw a total of 8 samples at 10 mL each.

Once the cannula has been inserted, the researchers will also collect a 10 mL sample of expired air to establish a baseline measurement of $^{12}C/^{13}C$ ratio.

Once you have rested for 30 minutes, a surgeon will take a muscle biopsy sample (please refer to the muscle biopsy section below for more detail on this procedure) from your thigh to allow for quantification of muscle glycogen, lipids and fatty acids.

Two muscle biopsy samples will be taken during each experimental trial, one before and one after exercise.

The process will involve a surgeon applying a local anaesthetic to the biopsy site, then making a small cut in the skin and removing a small amount of muscle tissue with a muscle biopsy needle. The incision will be dressed following removal of the muscle tissue to prevent infection.

As the muscle sample is classified as human tissue, its removal, storage and disposal comes under the Human Tissue Act 2004, and the associated code of practice issued by the Human Tissue Act Authority. You will be asked to provide written informed consent for your samples to be analysed for muscle glycogen, lipid content, enzyme activation and the protein content of lactate, fatty acid and glucose transporters. You will also need to confirm that you are happy for your muscle samples to be transported and stored until analysis at St James's University Hospital, Leeds under their Human Tissue Act License. A copy of your signed informed consent form needs to be stored with your muscle tissue samples, as this is a requisite of St James's University Hospital, Leeds transfer and storage policy.

Following the biopsy you will be allowed 10 to 30 minutes (or longer if required) of recovery before commencing the exercise test.

The exercise test will involve either cycling or running at constant intensity, (77% of your individual VO_{2max}) for 90 minutes whilst consuming a carbohydrate drink or water similar in flavour containing no carbohydrate (control).

The carbohydrate drink will be labelled by adding carbon 13 to the glucose, this naturally occurring isotope will allow us to follow the path of the ingested glucose through your energy systems telling us more about what fuels you are using whilst exercising.

You will not be told which drink you have been given in each trial, if you wish to know this information it will be provided to you upon completion of all of your experimental trials.

The drink will be given as 300 mL at the start of exercise, followed by 150 mL every 15 minutes, giving a total fluid intake of 1200 mL over a 90 minute period.

Following the completion of the constant intensity period of cycling or running, a second, post-exercise, muscle biopsy will be taken following the same procedures as the pre-exercise biopsy.

After a period of recovery you will undertake a 16 km cycling time-trial or 6 km running time-trial as quickly as possible.

You will complete a total of 4 experimental trials, (cycling with carbohydrate, cycling without carbohydrate, running with carbohydrate, and running without carbohydrate), and 2 VO_{2max} tests (1 cycling and 1 running). The order of the experimental trials will be randomly assigned.

How often will the tests be?

Each test will be separated by a minimum of 7 days. You will undertake the two VO_{2max} tests in consecutive weeks, then you will conduct your first two experimental trials over the following two weeks. Muscle biopsies will be taken from alternating legs in these first two experimental trials.

There will then follow a two week rest period to allow full recovery of both legs from the muscle biopsy samples.

The final two experimental trials will be conducted on consecutive weeks, again alternating between legs for the muscle biopsy samples.

What are the benefits of participating?

This study is being undertaken for research purposes to provide us with a better understanding of how the body regulates the use of carbohydrate and fat when carbohydrate beverages are consumed during cycling and running. This information will allow the development of specific carbohydrate ingestion strategies for cycling and running. You may benefit if you are interested in optimising your re-fuelling strategy to improve your triathlon performance. The findings from this study will help you to identify the best strategy for ingesting carbohydrate in both running and cycling to improve performance in each discipline.

You will also receive feedback on your VO_{2max} values, specific to cycling and running, which may help to optimise your training intensities, and feedback from the analysis of your food diary which may suggest some nutritional strategies to help improve your performance.

Upon completion of the full trial you will receive a £50 voucher for sportshoes.com.

What happens if something goes wrong?
All of the experimental procedures that will be undertaken in this study have been rigorously tested to ensure that they meet health and safety standards. All tests are routinely performed on volunteers and are undertaken by researchers who are trained and skilled to do so.

In the unlikely event that you do experience any problems as a results of your participation in this study, you must contact the lead researcher Alistair Black immediately (contact details at the end of this form). If you should be harmed as a result of your participation in this study, the University maintains indemnity insurance, the terms of which state that it will deal with cases only where the University has been deemed legally liable for incidents that occur as a direct result of the study.

Will my participation be confidential?
All information about you will be kept strictly confidential, your data will be coded and only the lead researcher will have access to the identification of each code.

A copy of your signed consent form needs to be stored with your muscle tissue samples and will be kept confidential at St James's University Hospital, Leeds under the terms of their transportation and storage policy in line with their Human Tissue Act License.

What will happen to the results of the study?
Once completed, the study will be written up as part of a doctoral thesis, and may also be submitted for peer reviewed publication in a relevant journal. Undergraduate and postgraduate students assisting with the collection of your data may use some of the anonymised data to complete their own research studies. All data will be anonymised as reported as average values rather than presenting individual data. The findings of the study will also be made available to interested participants.

Muscle Biopsies:

A muscle biopsy is the removal of a small piece of muscle tissue which will be examined for glycogen, lipid content, enzyme activation and the protein content of lactate, fatty acid and glucose transporters. The biopsy will be performed by Prof. Ernest Schilders, consultant orthopaedic surgeon or Mr Paul Parker, consultant orthopaedic surgeon.

How is the test performed?
The procedure will take place in the Carnegie Research Institute at Leeds Beckett University whilst you are awake.

The surgeon will apply a local anaesthetic to the biopsy area.

An open biopsy involves making a small cut in the skin, a needle is then inserted and muscle tissue is removed.

The muscle biopsy will be taken from the vastus lateralis (part of the quadriceps muscle group).

How do I prepare for the biopsy?
No special preparation is needed.

How will the test feel?
During the biopsy, there is usually little or no discomfort, you may feel some pressure or a 'tugging' sensation.

The anaesthetic may burn or sting when injected (before the area becomes numb). After the anaesthetic wears off the area may be sore for about a week.

What are the risks?
The risks are small, but may include:

- Bleeding
- Bruising
- Damage to the muscle tissue or other tissues in the area (very rare)
- Infection

All biopsy procedures will be performed by surgeons qualified and experienced in the procedure. Should you have any adverse reaction to the procedure or feel unwell during the process first aid will be available throughout from qualified first aiders, in addition to care provided by the surgeon undertaking the procedure.

If following the procedure you develop any pain and/or redness around the biopsy wound, or the wound discharges, no matter how minor you may feel this is, you can contact Professor John O'Hara immediately, either via phone: 0113 8125 239 or via e-mail: j.ohara@leedsbeckett.ac.uk, and he will make arrangements for the surgeon who took your biopsy to contact you. The surgeon who took your biopsy will then advise you of the best course of treatment based on your symptoms. If the issue remains unresolved or there are continuing complications following the biopsy, Professor John O'Hara will organise an appointment for you with the person who took your biopsy.

Care of the biopsy site;
Although you may exercise and return to your normal routine as soon as you feel able following the muscle biopsy you are advised to take care of the wound. We advise not submerging the wound in water for a minimum period of 48 hours following the biopsy, this includes swimming and taking a bath. You may shower, but you must keep the wound site dry by covering the dressing with plastic (cling film is good for this purpose). Do not rub the skin around the wound to dry it following a shower, instead gently pat the area dry with a clean towel. You will be reminded of this advice following each biopsy in addition to answering any questions you may have about wound care for specific personal circumstances.

You are welcome to bring one chaperone with you to make you feel more comfortable with this procedure should you wish to do so.

You maintain the right to withdraw at any point before, during or after the biopsy procedure has taken place.

The muscle sample is classified as human tissue, and as such its removal, storage, and disposal comes under the Human Tissue Act 2004, and the associated code of practice issued by the Human Tissue Act Authority. You therefore need to provide consent to the following:

- Provide written informed consent for all muscle biopsy samples to be analysed for glycogen, lipid content, enzyme activation and the protein content of lactate, fatty acid and glucose transporters.
- You will need to confirm that you are happy for your muscle tissue samples to be transported and stored at St James's University Hospital, Leeds under their Human Tissue Act License.
- Provide consent to a copy of your signed informed consent being stored with your muscle tissue samples, as this is a requirement of the transfer and storage policy.

Contacts:
If you require any further advice or information relating to this study at any point during your participation, you may contact the lead researcher Alistair Black, or members of his PhD supervisory team; Professor John O'Hara (Director of Studies), Dr Oliver Wilson (Supervisor), or Professor Roderick King (Supervisor). Alternatively for independent advice you may contact the Local Research Ethics Co-ordinator Karen Hind.

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Appendix C

Food, Fluid and Activity Record

Please complete the following details:

Name: .....

Age: .....

Weight: .....

Height: .....

Date of Birth: .....

Instructions

Keeping a record of what you normally eat and drink enables the dietitian to calculate your current daily food intake. Therefore subsequent nutritional information may be tailored to your specific requirements.

- Record **everything** you eat and drink over a three day period.
- Do not alter the food and fluid you normally consume.
- Record the time at which the food or fluid was consumed.
- Record all food eaten **as soon as possible after it is consumed**.
- Ideally the food record charts should be close at hand at all times, as reliance on memory increases error.
- List the foods in the order in which they are usually eaten. Note beverages as usually stated last, at the end of each meal.
- Nobody is going to criticise what you eat, so be honest. Do not leave out any wine/beer/spirits or any sweet/savoury snacks, as these will contribute significantly to your overall energy intake.
- It is important to record any **supplements** taken, i.e. Creatine, H5, vitamin/mineral complexes etc.
- Record the amount i.e. 1 tablet/200 mg, and when you take the supplement in the table provided.

Include as much detail as possible about the food consumed

- It is essential to include the amounts/portion size of the food actually eaten. This can be achieved by using household measures i.e.
    - Teaspoon/dessertspoon/tablespoon/ice-cream scoop
    - Small/medium/large
    - Portions/glass
    - ¼, ½, 1 pint
    - Thick/thin spreading
- If you cannot make a reasonable estimate of a food weight, describe the food size as accurately as possible, e.g. 2 x low fat pork sausages, 1 inch in diameter, 3 inches long, rather than guessing the weight.
- If consuming pre-packaged foods, i.e. a packet of crisps, if possible try to look at the packet and record the actual weight.
- Record anything added to drinks, e.g. sugar, milk etc.
- **Specify the type of food e.g.**

    Bread:
    - small/medium/large loaf
    - Thin/medium/thick slice
    - White/brown/Granary

    Milk:
    - Skimmed/semi-skimmed/full-fat
    - Pasturised/sterilised/UHT
- If known include the brand name i.e. Kellogs cornflakes, Del Monte fresh unsweetened orange juice.
- If possible keep food labels for reference.

Instructions (Continued)

- **Specify the cooking method**
    - It is important to record the cooking method as this makes a difference to the overall energy content of the diet.

        i.e. raw/boiled/poached/smoked/grilled/shallow fried/deep fried/braised/roasted

    - Try to include recipes for all homemade dishes of possible.
- If you record a food item/meal but only eat a fraction, remember to record the actual amount eaten e.g. ¼, ½ etc.
- Note anything you do not eat, e.g. jacket potato (not skin), chicken (not skin).

Food Record Chart (Example)

Day: ..... Date: .....

Time	Description of Food or Fluid	Weight or Household Measure	Cooking Method
Breakfast			
6:00 am	Del Monte unsweetened fresh orange juice	¼ pint	
	Kellogg's cornflakes	1 x med bowl	
	Granulated sugar	2 x tsp	
	Semi-skimmed milk (pasteurised)	1 x sm glass	
9:30 am	Streaky bacon	3 x rashers	Grilled
	Pork sausages	2 x (4" x 1")	Grilled
	Scrambled egg	Med portion	Microwave
	Large, medium sliced wholemeal toast	2 x slices	Toasted
	Thin spreading anchor butter	1 x knob	
	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tbsp.	
Mid-Morning			
11:00 am	McVities large chocolate digestive biscuits	x 3	
	Mug of coffee	x 1	
	Semi-skimmed milk (pasteurised)	1 x tbsp.	
	Granulated sugar	1 x tsp	
Lunch			
1:00 pm	Heinz country vegetable soup (tinned)	1 x med bowl	
	Crusty granary roll	2 x small	
	Cheese & ham sandwich containing:	x 1	
	Large medium sliced white bread	2 x slices	
	Mild cheddar cheese	1 x matchbox size	
	Thickly cut honey roast ham	1 x bread size	
	Hellman's mayonnaise	1 x tsp	
	Ski Bio (reduced fat) peach/mango yoghurt	1 x 150g pot	
2:30 pm	Banana	1 x med	
	Blackcurrant squash made with soda water	1 x pint	
Mid-Afternoon			
4:00 pm	Packet Seabrook cheese & onion crisps	1 x 130g pack	
	Kit-Kat (4 bar)	x 1	
	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tbsp.	
Evening meal			
6:00 pm	Steak & Kidney pie (individual, round with puff pastry top)	x 1 (6" x 6")	
	Jacket potato	1 x large	Baked
	Anchor butter	1 x knob	
	Carrots	Med portion	Boiled
	Rhubarb & apple crumble	Med portion	
	Bird's custard made with semi-skimmed milk	130 ml	
7:00 pm	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tbsp.	
Supper			
10:00	Jacob's cream crackers	x 4	
	Mild cheddar cheese	1 x matchbox size	
	Flora light	1 x knob	
	Mug of Cadbury's chocolate break	1 x 4tsp	
	Semi-skimmed milk (pasteurised)	1 x tbsp.	

Food Record: Day 1

Day: .... Date: ....

Time	Description of Food or Fluid	Weight or Household Measure	Cooking Method

Supplements Taken

Time Taken	Description	Amount

Food Record: Day 2

Day: .... Date: ....

Time	Description of Food or Fluid	Weight or Household Measure	Cooking Method

Supplements Taken

Time Taken	Description	Amount

Food Record: Day 3

Day: .... Date: ....

Time	Description of Food or Fluid	Weight or Household Measure	Cooking Method

Supplements Taken

Time Taken	Description	Amount

Appendix D

GI Questionnaire

Please rate each category on the scale from 1 to 10, with 1 being not at all, and 10 being extremely severe.

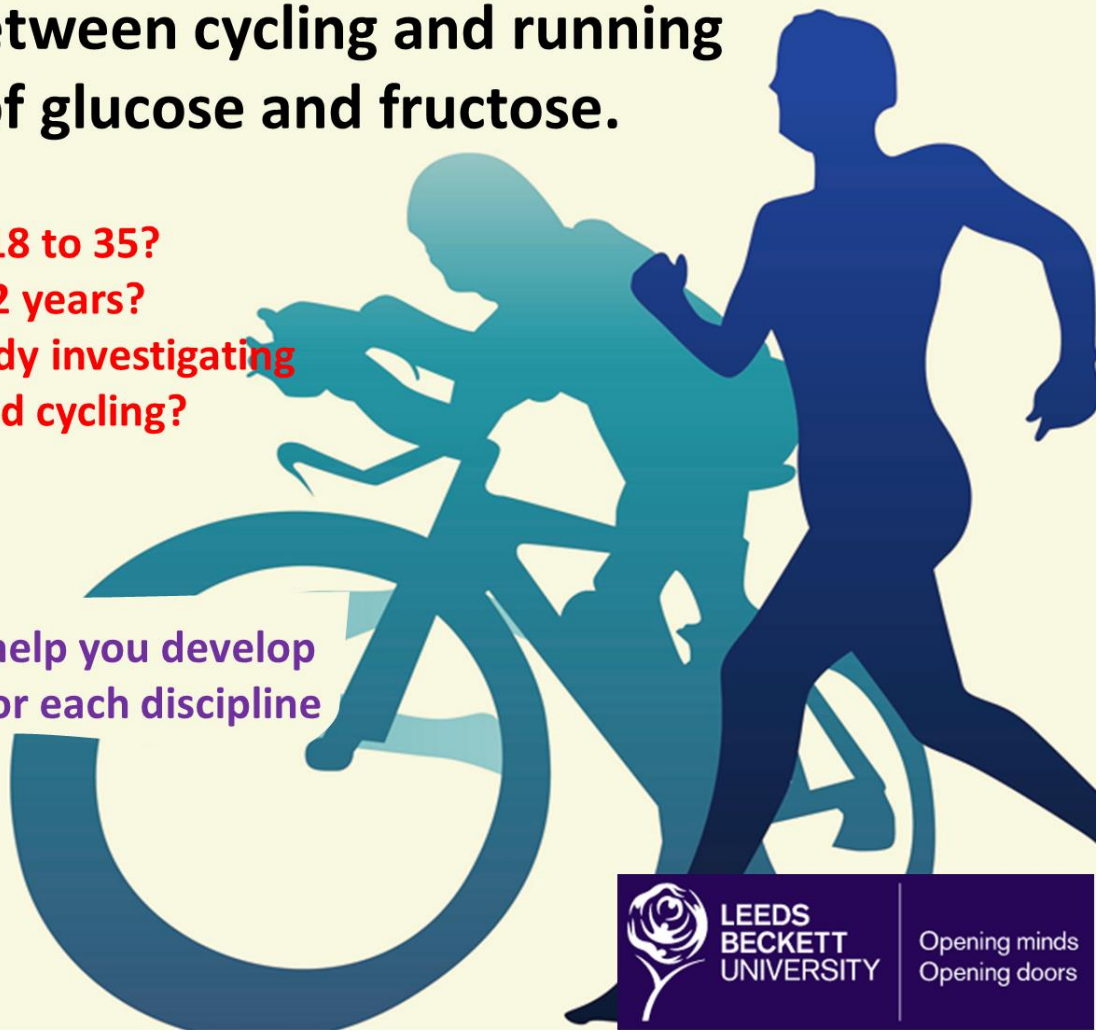
Tick the box to score each item.

Dizziness	1	2	3	4	5	6	7	8	9	10
Headache	1	2	3	4	5	6	7	8	9	10
Flatulence	1	2	3	4	5	6	7	8	9	10
Urge to Urinate	1	2	3	4	5	6	7	8	9	10
Urge to Defecate	1	2	3	4	5	6	7	8	9	10
Belching	1	2	3	4	5	6	7	8	9	10
Stomach Burn	1	2	3	4	5	6	7	8	9	10
Urge to Vomit	1	2	3	4	5	6	7	8	9	10
Side Ache (Left)	1	2	3	4	5	6	7	8	9	10
Side Ache (Right)	1	2	3	4	5	6	7	8	9	10
Stomach Problems	1	2	3	4	5	6	7	8	9	10
Nausea	1	2	3	4	5	6	7	8	9	10
Bloatedness	1	2	3	4	5	6	7	8	9	10
Stomach Cramps	1	2	3	4	5	6	7	8	9	10

Appendix E

A comparison of fuel use between cycling and running following the co-ingestion of glucose and fructose.

- **Are you a keen male triathlete aged 18 to 35?**
- **Have you been triathlon training for 2 years?**
- **Would you like to participate in a study investigating your fuel utilisation when running and cycling?**



As a participant you will receive:

- **A 3 day dietary analysis**
- **A VO_{2max} for cycling and running, to help you develop more specific training programmes for each discipline**
- **A £50 voucher for Sportsshoes.com**

For more information please contact:
Alistair Black
E-mail: Alistair.black@leedsbeckett.ac.uk
Tel: 0113 812 1877

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Opening minds
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Appendix F

Consent Form

Title: Substrate oxidation and endurance performance, the effects of ingesting multiple transportable carbohydrates in high intensity endurance cycling compared to running.

		Please Delete as Appropriate
1	I have read the Participant Information Sheet for this study.	**Yes/No**
2	I have had the opportunity to ask questions and discuss the research study.	**Yes/No**
3	I am satisfied with the answers to my questions.	**Yes/No**
4	I have received enough information about this study.	**Yes/No**
5	I understand that all data collected throughout this study will be kept safely and securely and will remain anonymous.	**Yes/No**
6	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason or prior notification.	**Yes/No**
7	I know how to withdraw from this study.	**Yes/No**
8	I understand that on request any personal data will be withdrawn from the study database should I wish to withdraw my participation.	**Yes/No**
9	I consent that my personal data can be retained in a database by the study investigators for the purposes of research and statistical analysis. These data may be made available with my anonymity protected to research students for the purposes of fulfilling their research projects.	**Yes/No**
10	I understand that the data collected as a result of my participation in this study can be published in academic/professional journals, and may also be presented at conferences. My anonymity will be protected at all times and no names will be included in any publications.	**Yes/No**
11	I agree that any personal information relating to me will be kept in a locked filing cabinet at Leeds Beckett University and will be destroyed after 10 years of the completion of the study.	**Yes/No**
12	I agree to participate in full, and understand all of the responsibilities and requirements of me as outlined in the participant information sheet.	**Yes/No**
13	I understand that any health risks or abnormalities found as a result of participation in this study may lead to my withdrawal and I may be asked to consult my GP.	**Yes/No**

14	I have read and understood the information sheet providing me with details on the procedures and risks associated with taking muscle biopsies.	**Yes/No**
15	I have been informed that muscle samples are classified as human tissue, and its removal, storage and disposal comes under the regulation of the Human Tissue Act 2004, and the code of practice issued by the Human Tissue Act Authority. I understand that I am providing written informed consent for each sample to be analysed for glycogen, lipid content, enzyme activation and the protein content of lactate, fatty acid and glucose transporters.	**Yes/No**
16	I consent to receiving a muscle biopsy procedure.	**Yes/No**
17	I am aware and give my consent for my muscle tissue samples to be transported and stored until analysis at St James's University Hospital, Leeds under their Human Tissue Act License.	**Yes/No**
18	I am aware and give my consent that a copy of my signed informed consent needs to be stored with my muscle tissue samples, this is a requirement of the transfer and storage policy.	**Yes/No**
19	I agree to take part in this research study.	**Yes/No**

Signature: .....

Name (Block Capitals): ..... Date: .....

Signature of person taking consent: .....

Name (Block capitals): ..... Date: .....

Appendix G

Pre-test instructions

Title: Substrate oxidation and endurance performance, the effects of ingesting multiple transportable carbohydrates in high intensity endurance cycling compared to running.

7 days before the test:

Avoid eating foods with a high carbon 13 content, these are mainly food derived from corn, and include;

Cornflakes	Sugar-cane products	Corned beef
Nachos/tortillas	Pepsi (including diet versions)	
Corn Starch	Coca Cola (including diet versions)	
Corn Syrup	Artificial sweeteners (sucralose, asprtamine)	
Popcorn	Corn-fed meats (e.g. corn-fed chicken)	

Train as normal

2 days before the test:

Continue to avoid carbon 13 foods (see above)

Avoid drinking alcohol

Avoid high intensity exercise – focus on technique rather than intensity or endurance for the first day, and try to make the second day (the day before the test) your rest day.

1 day before the test:

Eat the food provided for you – tick off foods on the list provided as you eat them, and make a note of any additional foods or supplements that you consume.

Do not eat for 10 hours before your test – e.g. if your test is a t 6 am do not eat after 8 pm the night before.

You are encouraged to still drink plain water in this 10 hour fasting period, we would suggest that you aim to drink approximately 500ml of plain water over a 2 hour period before you go to bed.

Test day:

Get up and drink plain water as you feel.

Get together any kit you will need for the test – we request that you use the same kit for each test, and would therefore suggest for your comfort that you wear your triathlon kit (tri-shorts & singlet or tri-suit). Bring a towel and wash kit (shower facilities are available). Your choice of clothing for your journey home after the test.

When you arrive at the laboratory you will be guided through everything you need to do for the test.

We would advise that you do not undertake any additional training this day following the test.

Appendix H

The Leeds Teaching Hospitals NHS
NHS Trust

Dr O Rotimi
Consultant Histopathologist
Department of Histopathology
Bexley Wing, Level 5
St. James's University Hospital
Beckett Street, Leeds
LS9 7TF

Secretary: 0113 2067578
Direct line: 0113 2067786

25th April 2016

Dr. Oliver Wilson,
Senior Lecturer,
School of Sport,
Leeds Beckett University,
City Campus,
Leeds,
LS1 3HE.

Dear Dr. Wilson,

Re: Storage of human skeletal muscle biopsies.

St James University Hospital's Department of Histopathology agrees to store the Leeds Beckett University School of Sport and Exercise Science's human skeletal muscle biopsies. Storage will be in accordance with the requirements of the Human Tissue Act 2004 and the associated Code of Practice issued by the Human Tissue Authority.

The following caveats are stipulated:

1. Access to the facility can only be during working hours (9am - 5pm) on week days
2. The freezer is alarmed to detect malfunction, but we will not accept any liability in the event of power failure or malfunction of the freezers
3. A storage charge of £50 per month will apply

Signed: Date: 25/04/2016

Print: Dr. Olorunda Rotimi
Departmental Lead for Research and Trials

Contact: 0113 2067786

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