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## **Reliability of a High-Intensity Endurance Cycling Test.**

**Abstract** This study assessed the reproducibility of performance and selected metabolic variables during a variable high-intensity endurance cycling test. Eight trained male cyclists (age:  $35.9 \pm 7.7$  years, maximal oxygen uptake:  $54.3 \pm 3.9 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) completed four high-intensity cycling tests, performed in consecutive weeks. The protocol comprised: 20 minutes of progressive incremental exercise, where the power output was increased by 5% maximal workload ( $W_{\text{max}}$ ) every 5 minutes from 70%  $W_{\text{max}}$  to 85%  $W_{\text{max}}$ ; ten 90 seconds bouts at 90%  $W_{\text{max}}$ , separated by 180 seconds at 55%  $W_{\text{max}}$ ; 90%  $W_{\text{max}}$  until volitional exhaustion. Blood samples were drawn and heart rate was monitored throughout the protocol. There was no significant order effect between trials for time to exhaustion (mean:  $4113.0 \pm 60.8$  s) or total distance covered (mean:  $46126.2 \pm 1968.7$  m). Total time to exhaustion and total distance covered showed very high reliability with a mean coefficient of variation (CV) of 1.6% (95% Confidence Intervals (CI)  $0.0 \pm 124.3$  s) and CV of 2.2% (95% CI  $0.0 \pm 1904.9$  m), respectively. Variability in plasma glucose concentrations across the time points was very small (CV 0.46% to 4.3%, mean 95% CI  $0.0 \pm 0.33$  to  $0.0 \pm 0.94 \text{ mmol} \cdot \text{L}^{-1}$ ). Plasma lactate concentrations showed no test order effect. The reliability of performance and metabolic variables makes this protocol a valid test to evaluate nutritional interventions in endurance cycling.

## Introduction

To evaluate the metabolic attributes and performance benefits of different nutritional interventions such as carbohydrate ingestion prior to and/or during exercise, it is important to use an appropriate valid and reliable exercise test protocol. Such test protocols, used within appropriate experimental designs, enable researchers to test specific research hypothesis with greater confidence that any differences (metabolic and/or performance) are real. Traditionally time to exhaustion protocols have been used, where a sub-maximal intensity set as a percentage of maximal oxygen uptake ( $\dot{V}O_2$  max) or maximal power output ( $W_{max}$ ) is maintained until exhaustion. This type of protocol has been shown to have poor reliability, with coefficients of variation (CV) typically >10% [16, 20, 22, see 5 for a review) and have been suggested to lack ecological validity [5, 29, 36]. More recently there has been an increased use of time trial protocols, where a set amount of work or a set distance is completed as fast as possible. These types of protocols have been shown to have high reliability with typical CV <5% [6, 16, 33, 34, see 5 for a review) and are considered to be more ecologically valid, as they are more applicable to real-world competition [6], as competitors generally strive to complete a given distance in the minimum time.

There is much continued debate over protocol design. However, the selection of the protocol is dependent upon the research question(s) being addressed. For example, if metabolic aspects of fatigue are to be thoroughly assessed, then time trial protocols may not be appropriate. Time trial protocols are likely to produce variability in exercise intensity between trials as per competitive cycling, whereas time to exhaustion protocols provide a controlled environment for comparisons of metabolic variables between trials [5]. The majority of time trial protocols use a pre-load prior to the time trial to try and overcome this issue, however they are of short duration, between 20 to 45 minutes [14, 15, 23] and are typically constant intensity exercise. The short duration of these pre-loads are important for those wanting to study energy supply as they may not produce sufficient glycogen depletion prior to the time trial. Furthermore, they are only likely to detect small and potentially crucial individual differences, but also may, lack adequate statistical power. However, time to exhaustion protocols are closely related to glycogen depletion [2]. Accordingly they are used as a measure of endurance capacity and may be more likely to detect differences during trials that influence metabolic aspects of fatigue. Therefore, it may be worth considering a protocol where the exercise intensity is controlled for a set amount of work over a longer duration (greater than one hour) than has been previously used within the literature [14, 15, 16, 23].

The ecological validity of the protocol should also be considered and competitive endurance cycling is normally characterized by high-intensity efforts interspersed with sustained steady state exercise [8, 26, 27, 28, 37]. This race and training situation is not always reflected in laboratory tests, such as those previously mentioned, which will produce different metabolic responses in comparison to more constant load tests [28]. The selection of a variable high-intensity protocol is likely to challenge glycogen depletion in both type I and type II muscle fibres [25, 38].

It is clear that a variable intensity cycling protocol to be ultimately used on nutritional intervention studies to maximize endurance capacity differences between conditions that notably influence metabolic aspects of fatigue, particularly those that relate to carbohydrate metabolism and glycogen depletion, is highly desirable.

Therefore the aim of this study was to assess the test re-test reliability of our newly designed variable high-intensity cycling test under standardized conditions. The study also assessed the test re-test reliability of plasma glucose and lactate concentrations. This was deemed relevant as there is considerable interest in the maintenance or modulation of plasma glucose concentrations during exercise, as well as that arising from either pre and during exercise ingestion of carbohydrates in situations that may be relevant for competitive performance.

## **Methods**

### **Participants**

Eight trained male cyclists (mean: age  $35.9 \pm 7.7$  years, body mass  $75.5 \pm 7.6$  kg, body fat percentage  $13.4 \pm 3.9$ ) were recruited to take part in this study. The cyclists had trained regularly for the last 5 years for up to 15 hours per week. They were competitive club level road racers, which is reflected in their  $\dot{V}O_2$  max ( $54.3 \pm 3.9$  mL·kg<sup>-1</sup>·min<sup>-1</sup>) and they were in a maintenance phase of training throughout the study. Written informed consent was obtained prior to participation in the study. The protocol employed during this investigation received ethical approval from the research ethics committee of the local Teaching Hospitals NHS Trust and was performed in accordance with the ethical standards specified by Harris and Atkinson [10].

### **Experimental Design**

The repeatability of the protocol was assessed in a single blind (participants) study, which consisted of a set amount of work followed by continuous cycling to volitional exhaustion. Each cyclist completed a familiarisation ride to allow habituation to the laboratory equipment and procedures employed in this study. A

week later the cyclists then completed a maximal incremental work load test to exhaustion to assess their  $W_{max}$  at  $\dot{V}O_2$  max. This was followed a week later by the experimental protocol, which involved the cyclists performing four exercise tests, one week apart.

#### Familiarisation Ride

The cyclists performed the test on a standardized adjustable road bicycle (TREK 1400, Stif Cycles, Headingley, UK) fitted with Schoberer Rad Messtechnik (SRM) Powermeters (SRM, Germany), mounted on an air braked cycle ergometer (Kingcycle® Ltd, High Wycombe, U.K.). The Kingcycle® was calibrated as described by Schabert et al. [29]. The SRM Powermeters (SRM, Germany) were calibrated prior to the study and enabled high precision (<0.5 %) measurements of power output (W) and was used to measure the cyclists power outputs. This equipment was used for the maximal work load test and experimental trials.

The familiarization ride consisted of three 10-minute continuous progressive increments of cycling which were completed at 100 watts (W), 150 W and 200 W, followed by two 5 minute continuous progressive increments, completed at 225 W and 250 W, followed by five 90 seconds sprints at 300 W separated by 180 seconds recovery at 200 W. This was followed by a 5 minute period, where the cyclists completed as much work as possible.

#### Maximal Work Load Test.

At least one week prior to the experimental trials, the cyclists performed a continuous incremental cycling test to exhaustion to assess  $W_{max}$  [(W) mean:  $342.2 \pm 37.5$  W] as previously described by Kuipers et al. [19]. There are a variety of methods to prescribe relative exercise intensity during experimental trials. This study used percentages of the  $W_{max}$  to determine the workloads to be undertaken during the experimental trials (e.g. power output (W) at a given %  $W_{max}$ ), a method typically used within the nutritional intervention literature [13, 15, 18].

#### Experimental Exercise Trials

The cyclists were asked to record their food intakes and activity patterns during the three days prior to the first experimental endurance test. They were then instructed to repeat this diet and activity pattern for the next trials. Each of the four reliability trials took place following an overnight fast of at least 12 hours, typically associated with studies evaluating the effects of carbohydrate ingestion on endurance performance/capacity [15,

35], between 0600 and 0900. Each cyclist started their experimental trials at the same time of day, to avoid any influence of circadian variance. Cyclists were instructed to refrain from any strenuous activity, alcohol or caffeine consumption in the previous 24 hours. Upon arrival, an 18-gauge catheter (Biovalve Catheter, Vygon, Gloucestershire, UK) was inserted into an antecubital vein. A catheter extension with bionecteur 2 and clamp was fitted to the catheter (Vygon, Gloucestershire, UK). The extension was kept patent by flushing with 1.0 to 1.5 ml of isotonic saline (Becton Dickinson, UK) after each sample collection. Resting blood samples were drawn, at -5 minutes (15 minutes after the insertion of the catheter) and -2 minutes prior to fluid consumption and analyzed for plasma glucose and plasma lactate concentrations.

The cyclists then consumed 1 litre of lemon and lime flavoured water with electrolytes (607 mg sodium and 117 mg of potassium) within a 10 minute period, 30 minutes before starting the experimental cycling test. The timing of ingestion was to mimic previous protocol designs [15, 18, 21], which have evaluated the ingestion of carbohydrate within the hour before exercise on metabolic and endurance performance variables, which this protocol would ultimately assess.

This was followed by 20 minutes of recumbent rest. Blood samples were drawn at 13, 18, 23 and 28 minutes after the initial fluid consumption and analyzed for plasma glucose and plasma lactate concentrations. Following this, the cyclists then completed an initial 2 minute warm-up period, to enable them to build up to the starting workload, followed by four 5 minute continuous progressive increments of cycling completed at a workload corresponding to 70%, 75%, 80% and 85%  $W_{max}$ . This was followed by ten 90 second sprints at a workload corresponding to 90%  $W_{max}$ , separated by 180 seconds recovery at a workload corresponding to 55%  $W_{max}$ . Blood samples were drawn at the end of each 5 minute stage during the initial 20 minutes, and the end of each 180 second recovery phase. Heart rate was monitored at the end of each workload by a radio telemetry heart rate monitor (Polar Vantage NV, Kempe, Finland). See figure 1 for a schematic representation of the experimental protocol.

If a cyclist completed all 10 sprints, following a three-minute interval at 55%  $W_{max}$ , a capacity trial to fatigue was undertaken at 90%  $W_{max}$ . Exhaustion during the 10 sprints at 90%  $W_{max}$  or the capacity trial at 90%  $W_{max}$  was defined as an inability to maintain power output within 5 W of that expected and an inability to restore this within 15 seconds despite verbal encouragement. No feedback on elapsed time was provided, as this would have introduced potential bias with cyclists targeting previous times. The power output during the experimental trials was controlled by the Powermeter display, with the cyclists using a pre-defined cadence,

Gear selection for specific power outputs, was established during the initial trial, recorded and replicated during the three remaining trials.

#### Blood Sampling and Analysis

Blood samples drawn for plasma glucose and lactate concentrations were collected in fluoride oxalate containing tubes (Becton Dickinson, UK), kept on ice and analyzed the same day by the department of Chemical Pathology, Leeds General Infirmary. Plasma glucose concentrations were determined by the Glucose Oxidase method, which is based on the modified method of Keston [17]. Plasma lactate concentrations were determined by an enzymatic assay [31]. Samples were run on an ADVIA Centaur® System (Bayer Diagnostics, Newbury, Berks, UK).

#### Statistical Methods

Data were approximated to a normal distribution (Shapiro Wilk test) and are presented as mean  $\pm$  standard deviation. Repeated measures ANOVA was used to establish whether there was an order effect between the four experimental trials. One-way ANOVA was used to analyze time to exhaustion and distance covered, while two-way ANOVA was used to analyze plasma glucose, plasma lactate concentrations and heart rate. Individual CVs were calculated for each cyclist and then averaged to obtain an overall CV for time to exhaustion and distance covered. CV for plasma glucose concentration and heart rate, was calculated for each time point. A test-retest CV lower than 3.5% was regarded as highly reliable [12, 16]. 95% confidence intervals for time to exhaustion, distance covered and plasma glucose concentrations were calculated using the method described by Bland and Altman [3]. Data were evaluated using SPSS for windows version 17 (Chicago, USA). A 0.95 level of confidence was predetermined to denote statistical significance ( $P < 0.05$ ).

#### Results

Table 1, panel A, shows the total time to exhaustion across the whole exercise test for the four trials completed by the eight cyclists. Individual CVs range from 0.52% to 2.82%, with a mean  $\pm$  SD value of  $1.61 \pm 0.8\%$ . Although there was an increase of 48 s in the mean time to exhaustion there were no significant differences across all four trials due to the variability within the group, which is reflected by the standard deviation which increased from 69 s to 107 s.

Panel B of Table 1 shows the time to exhaustion at 90% Wmax for the performance component of the test, which is, on average, 213 s (compared to 4113 s for the whole test). Given that this is the only variable component of the test the standard deviations for both panel A and B are identical for trial 1 to 4. Individual CVs range from 9.7% to 56.2%, with a mean  $\pm$  SD value of  $31.7 \pm 13.8\%$ .

Figure 2 shows the limits of agreement [2] graph for the variable component of the test, which is time to exhaustion at 90% Wmax. There is no systematic bias as the mean of the differences is zero. However, the magnitude of variability indicated by the 95% confidence intervals for the standard deviation of differences between trials is high in comparison to the mean time to exhaustion across all trials (mean  $\pm 1.96 \times$  SD differences for trials 1 to 4 =  $0.0 \pm 124.32$  s; mean time to exhaustion =  $212.97 \pm 60.82$  s).

Table 2 shows the total distance covered by all cyclists in all trials (A), together with the distance covered during the prescribed period of the protocol (B), prior to the performance test at the end (C). No significant differences between the trials were observed. The variability between trials for each cyclist and for the group as a whole is very small when the distance covered for the set workloads is considered, as indicated by the range of individual CVs (0.68% to 1.83%). Even when the variation associated with the performance component of the test is included (A) the individual CVs remain very small (0.75% to 3.39%). In contrast, when individual between trial variation is assessed for the performance component in isolation, the CVs increase considerably. The limits of agreement for the total distance covered (95% CI  $0.0 \pm 1904.9$  m), the prescribed part of the protocol (95% CI  $0.0 \pm 881.5$  m) and the performance test show (95% CI  $0.0 \pm 1629.0$  m) no systematic bias for any of these data sets. Variability, as indicated by the confidence intervals, ranges from less than 2.5% for the prescribed part of the protocol up to 5% for the total distance covered.

Figure 3 shows the plasma glucose (A) and plasma lactate (B) responses to the exercise test. Plasma glucose concentrations significantly decreased (mean of  $0.38 \pm 0.05$  mmol·L<sup>-1</sup>, across all trials, ( $P < 0.05$ )) during the initial 15 minutes of exercise. Within 10 minutes they significantly increased to a relative hyperglycaemia ( $> 5.5$  mmol·L<sup>-1</sup>,  $P < 0.05$ ) until 51.5 minutes into the exercise test. Thereafter a return towards basal concentrations was seen, but they remained significantly ( $P < 0.05$ ) higher throughout the remainder of the exercise period compared to basal concentrations. The variability across the time points is very small, as indicated by the range of CVs (0.46% to 4.27%). There is no systematic bias for any of the time points and variability, as indicated by the range of confidence intervals (95% CI  $0.0 \pm 0.33$  mmol·L<sup>-1</sup> to  $0.0 \pm 0.94$  mmol·L<sup>-1</sup>), for the mean plasma glucose concentrations for each time point across all trials.

Plasma lactate concentrations significantly increased to a mean of  $8.6 \pm 0.5 \text{ mmol}\cdot\text{L}^{-1}$  ( $P < 0.001$ ) across all trials following the initiation of exercise, thereafter they significantly decreased to  $6.9 \pm 1.1 \text{ mmol}\cdot\text{L}^{-1}$  ( $P < 0.001$ ) and  $6.3 \pm 0.7 \text{ mmol}\cdot\text{L}^{-1}$  ( $P < 0.001$ ), for the remaining two samples points, respectively. There were no significant differences between trials for plasma glucose and plasma lactate concentrations.

Heart rates showed a predictable pattern of response consistent with exercise at different intensities. Over the initial 20 minutes of exercise, mean heart rate progressively increased from approximately  $141 \text{ b}\cdot\text{min}^{-1}$  to  $168 \text{ b}\cdot\text{min}^{-1}$ , with a magnitude of difference ranging between 4 to  $6 \text{ b}\cdot\text{min}^{-1}$  between trials. However, there were no significant differences between trials and the variability across the time points is very small, as indicated by the range of CVs (0.94% to 1.8%). The mean heart rate for each of the ten 90% efforts was  $168 \text{ b}\cdot\text{min}^{-1}$ , whereas it increased from  $141 \text{ b}\cdot\text{min}^{-1}$  to  $146 \text{ b}\cdot\text{min}^{-1}$  over the ten 55% recovery efforts, with a magnitude of difference ranging between 4 to  $6 \text{ b}\cdot\text{min}^{-1}$  between trials. However, there were no significant differences between trials and the variability across the time points is very small, as indicated by the range of CVs for the 90% (0.92% to 1.24%) and 55% efforts (0.95% to 2.02%).

## Discussion

The present study shows that this variable high-intensity cycling protocol is a highly reliable test, as indicated by the low CV (1.6%) between individual performances, for the assessment of the prescribed period of the protocol and including the time to exhaustion element (viz. total time to exhaustion) using SRM Powermeters. The CV of 1.6% for total time to exhaustion is comparable to other studies within the literature, which have used a pre-load (20 to 45 minutes of constant intensity cycling at 50% to 75%  $W_{\text{max}}$ ) followed by a time trial equivalent to 15 to 40 minutes [6, 16]. It is also more reliable in comparison to those studies that have only used time to exhaustion exercise tests where the CV is typically  $>10\%$  [5]. The total time to exhaustion using the protocol is therefore likely to be more reliable than straightforward time to exhaustion protocols. Furthermore, the low CV (1.6%) for total time to exhaustion allows the detection of worthwhile changes of 1% and 5%, with approximately 60 and less than 10 participants, respectively [1], during nutritional intervention studies which use two conditions in a repeated measures design.

The time to exhaustion at 90%  $W_{\text{max}}$  for the endurance capacity component of the test showed a higher CV (32%) and high individual variability in comparison to the total time, which is similar to the variability reported in the literature [16, 22]. However, this is the only variable aspect of the protocol, and the larger CV may be explained by the ratio of this performance component to the whole test being on average

approximately 1:20. Nevertheless, the good reliability of the prescribed period of the protocol is appropriate for evaluating the effects of nutritional interventions on metabolic variables, given the stability and reproducibility of data reported for plasma glucose and lactate concentrations, irrespective of the performance capacity test. While time to exhaustion at 90%  $W_{max}$ , though less reliable, in combination with the fixed amount of work allows discrimination between interventions that influence metabolic aspects of fatigue, which time trials are less suited to.

There was no systematic bias in total time to exhaustion between the four trials. This shows that there was not a learning effect or any other test order effect during the four trials. This may be explained by the tight control of power output during the prescribed period of the protocol. This implies that it is not necessary to perform an extra learning trial beyond a familiarisation session, before the start of a study using the described variable high-intensity cycling test, with cyclists who are familiar with laboratory procedures and the cycle ergometer being used.

The variable high-intensity cycling protocol is also a reliable test for the assessment of distance covered. The CV (2.2%) and 95% confidence intervals for total distance covered shows high reliability, as does the CV (1.2%) and 95% confidence intervals for the prescribed period of the protocol. This shows that the variations associated with the transitions between set workloads in the prescribed stages of the protocol have only a relatively minor effect on total distance covered. However, the trial variations for the performance component are considerably greater. Overall, the observed level of variability indicates that the protocol is fit for purpose for evaluating group differences between nutritional interventions which influence metabolic aspects of fatigue, being able to detect worthwhile changes of 5% with approximately 10 participants [1]. However, caution is needed when interpreting one-off individual responses due to individual variability reflected in the CV and 95% confidence intervals for the performance component of the protocol.

A novel aspect of this study was the assessment of the reliability of plasma glucose concentrations at rest and during prolonged variable high-intensity cycling. This paper is the first to show that plasma glucose responses under standardised conditions are consistent across repeated trials, being able to detect worthwhile changes within 5% from 10 to 20 participants, given the CV range (0.46% to 4.3%) [1]. This is by no means the case in all situations reported in the literature as it is well known that individuals may have very different intrinsic glucose sensitivity and variable glucose concentration response during specific protocols. Nevertheless, there are still perturbations in glucose concentrations throughout the different phases of the protocol reported here, but these are consistent between trials. It is important to both assess the repeatability and to characterize

and understand plasma glucose responses as this provides the researcher with confidence to make comparisons to any nutritional supplement likely to affect glucose concentrations. Therefore, researchers would be able to distinguish real differences in comparison to the normal patterns of response and associate these with the nutritional supplement consumed.

The slight decline in plasma glucose concentrations during the initial 20 minutes of exercise is comparable to that shown previously within the literature for steady state (65% to 72%  $\dot{V}O_2$  max) cycling [13, 30, 32], where placebo was consumed within the hour before exercise.

The decline in plasma glucose concentrations at the initiation of exercise is probably due to the effect of increased contractile activity on muscle glucose uptake. According to Short et al. [32] increased contractile activity is known to stimulate glucose transport protein (GLUT-4) translocation from the intracellular pools to the plasma membrane, increasing glucose uptake into the working muscle upon the initiation of exercise [7, 21]. In the present study the supply of glucose to the plasma would almost exclusively be from the liver, either by glycogenolysis or gluconeogenesis, and uptake by the muscle from plasma would at the onset of exercise be greater than this.

The protocol was specifically designed to challenge glucose homeostasis, during the initial 20 minutes of exercise, as increased exercise intensity is associated with increased carbohydrate utilisation [11] so the decline in plasma glucose concentrations was expected. The rationale for this design was that the investigators are interested in the occurrence of rebound hypoglycaemia, which has been shown to typically occur within 15 to 20 minutes after the start of exercise [4, 13, 14, 18, 21], following the pre-exercise ingestion of 75g of glucose 30 to 45 minutes prior to sub-maximal exercise (70 to 76%  $\dot{V}O_{2\text{ max}}$ ). The present study observed the maintenance of plasma glucose concentrations above basal concentrations (relative hyperglycaemia) during the repeated sprints. The demand for glycolytic substrates either from glycogenolysis or from plasma glucose uptake would almost certainly be greatest in fast twitch fibres [25] during this period of the protocol and may explain this relative hyperglycaemia. This maybe a consequence of an accelerated glucose production from the liver and/or the transient reduction in blood glucose uptake during the lower intensity period following each sprint and the difference in actual glucose uptake from the blood by different fibre types during glycogen depletion. It may also be expected that glycogen depletion will be relatively faster in the fast twitch muscle fibres during the series of high-intensity sprints. This relative hyperglycaemia has not been shown previously and is in contrast to other studies that have utilised high-intensity intermittent cycling protocols [9, 24, 39] where plasma glucose concentrations have more typically remained stable or declined over time. This may be

explained by the more frequent bouts of high-intensity cycling performed in this protocol in comparison to the existing literature. This, in conjunction with elevated plasma lactate concentrations, could be of advantage when investigating the proportionalisation of fuel utilization and overall carbohydrate utilization from sources such as liver and muscle glycogen, when exogenous glucose sources are introduced into the system.

The plasma lactate concentrations following the initial 20 minutes of progressively increasing intensity of cycling indicate the high-intensity nature of the exercise challenge producing values similar to those associated with maximal exercise. Thereafter the protocol facilitated some recovery in plasma lactate concentrations, allowing participants to continue, but maintained the challenge to carbohydrate as a fuel source for ATP synthesis, which is a strength of this variable high-intensity exercise protocol.

In conclusion, the present study has demonstrated that an ecologically valid variable high-intensity cycling protocol followed by a time to exhaustion endurance capacity test is a reliable measure of overall time to exhaustion and distance covered. The performance capacity test on its own is less reliable, but allows for the assessment of metabolic aspects of fatigue. A novel finding of the present study was the relative hyperglycaemia shown during the repeated bouts of high-intensity exercise and the fact that plasma glucose concentrations were consistent across trials. This protocol design provides a stable basis from which to compare the effects of different nutritional interventions on metabolic variables (plasma glucose and lactate) during the prescribed part of the protocol, and endurance capacity through the challenge of a prolonged (>1 hour) variable high-intensity endurance exercise test. Finally, the small CV's allow the detection of small-moderate worthwhile changes with practically feasible sample sizes between 10 and 60 participants.

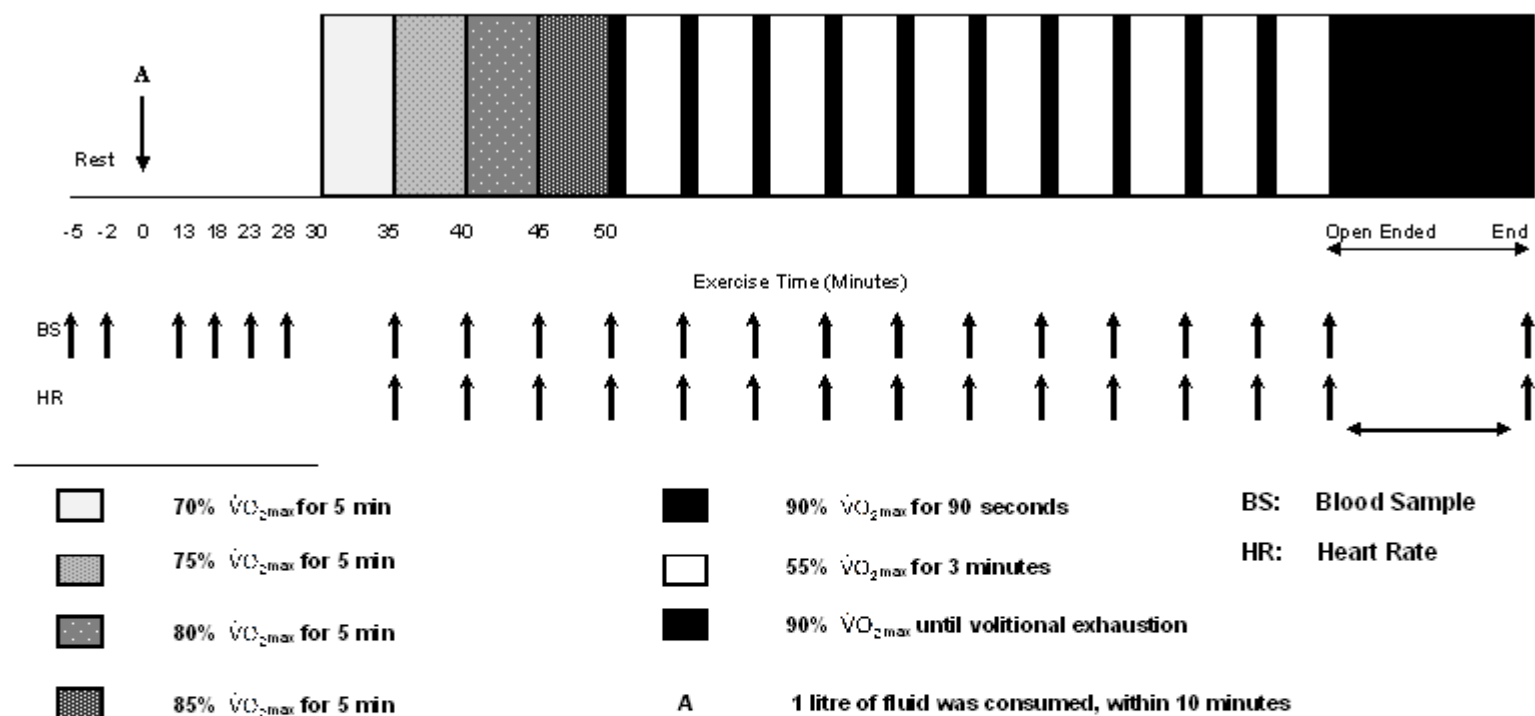
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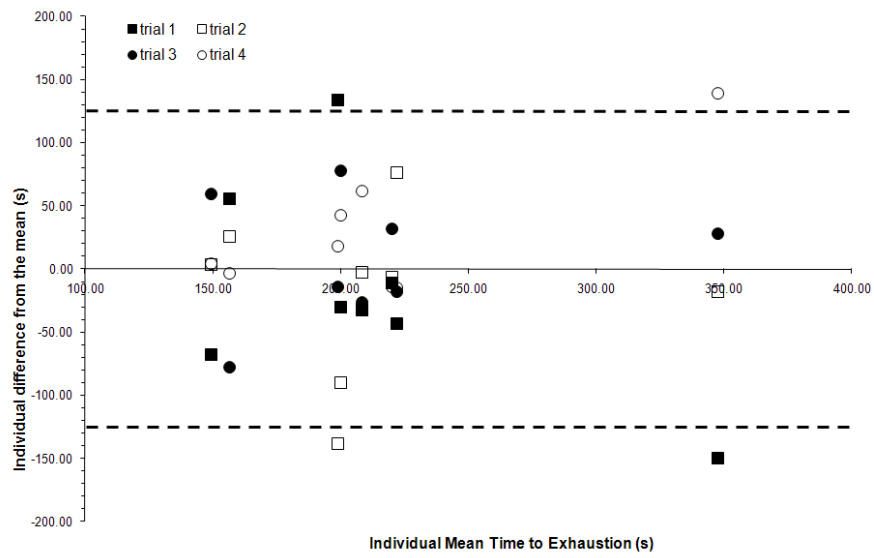
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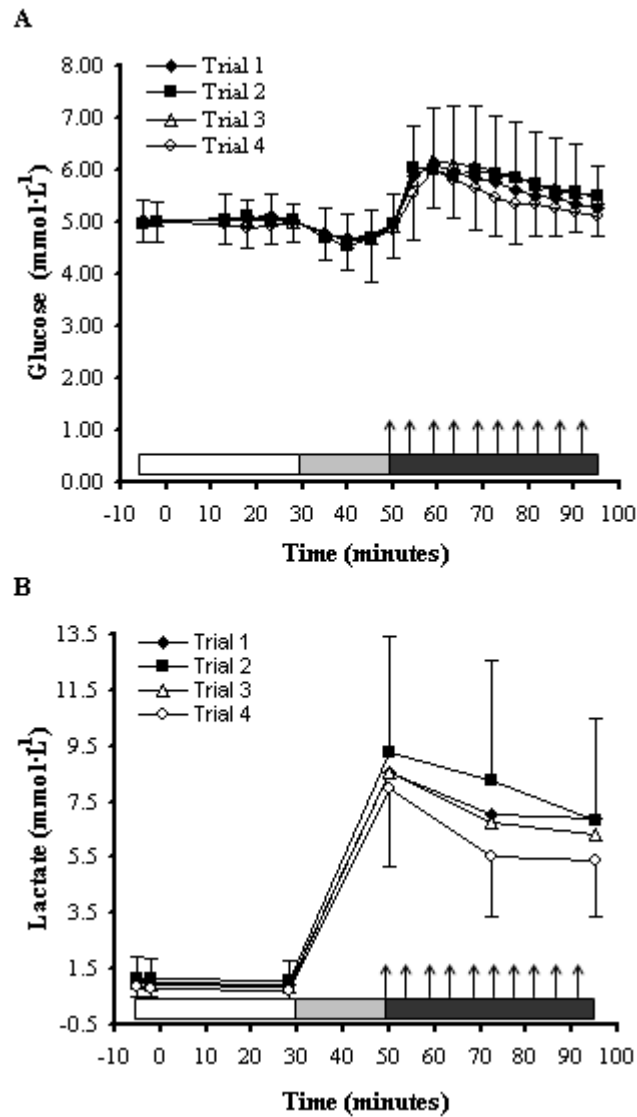
## Figures and Tables



**Figure 1** Schematic representation of the experimental cycling protocol. The timing of the pre-exercise blood samples is related to the cyclists performing a two minute warm up, so that the exercise protocol could be started exactly 30 minutes following the consumption of the electrolyte beverage. Hence timing of blood samples was set by working backwards from the start of the warm-up, with 5 minute intervals over a 15 minute time period from the 28 minute sampling time point.



**Figure 2 Limits of agreement for time to exhaustion at 90% Wmax.**



**Figure 3** Plasma glucose (A) and lactate (B) concentration responses to the variable high intensity continuous exercise protocol. Data are mean  $\pm$  SD. *White rectangle* indicates the rest period prior to the commencement of exercise; *grey rectangle* indicates 20 minutes of progressive intensity cycling from 70% to 85%  $W_{\text{max}}$ ; *black rectangle with arrows* indicates the ten 90 s sprints at 90%  $W_{\text{max}}$  interspersed with 180 s of active recovery at 55%  $W_{\text{max}}$ .

**Table 1. Total time to exhaustion (a) and 90% Wmax (b) until exhaustion.**

<b>(a)Total Time to Exhaustion (s)</b>							
<b>Cyclist</b>	<b>Trial 1</b>	<b>Trial 2</b>	<b>Trial 3</b>	<b>Trial 4</b>	<b>Mean</b>	<b>SD</b>	<b>CV%</b>
1	4076	4106	4082	4170	4109	43.0	1.05
2	3982	4053	4109	4054	4050	52.1	1.29
3	4079	4198	4104	4107	4122	52.2	1.27
4	4109	4114	4152	4106	4120	21.4	0.52
5	4112	4082	3979	4053	4057	57.0	1.41
6	4098	4230	4276	4387	4248	119.6	2.82
7	4233	3961	4085	4117	4099	111.8	2.73
8	4070	4010	4178	4143	4100	75.1	1.83
<b>Mean</b>	<b>4094.9</b>	<b>4094.3</b>	<b>4120.6</b>	<b>4142.1</b>	<b>4113.0</b>	<b>66.5</b>	<b>1.61</b>
<b>SD</b>	<b>69.2</b>	<b>89.8</b>	<b>85.9</b>	<b>106.6</b>	<b>60.8</b>		<b>0.80</b>
<b>(b) 90% Wmax until exhaustion (s)</b>							
<b>Cyclist</b>							
1	176.0	206.0	182.0	270.0	208.5	43.0	20.6
2	82.0	153.0	208.9	154.0	149.5	52.1	34.8
3	178.9	298.0	204.0	207.0	222.0	52.2	23.5
4	208.9	214.0	252.0	205.9	220.3	21.4	9.7
5	212.0	182.0	79.0	153.0	156.5	57.0	36.4
6	198.0	330.0	376.0	487.00	347.8	119.6	34.4
7	333.0	61.0	185.0	217.0	199.0	111.8	56.2
8	170.0	110.0	278.0	243.0	200.3	75.1	37.5
<b>Mean</b>	<b>194.9</b>	<b>194.3</b>	<b>220.6</b>	<b>242.1</b>	<b>212.9</b>	<b>66.5</b>	<b>31.7</b>
<b>SD</b>	<b>69.2</b>	<b>89.8</b>	<b>85.9</b>	<b>106.6</b>	<b>60.8</b>		<b>13.8</b>

**Table 2. Total distance covered (A), distance covered during the set work load (B) and 90% Wmax until exhaustion (C).**

<b>a. Total Distance Covered (metres)</b>							
<b>Cyclist</b>	<b>Trial 1</b>	<b>Trial 2</b>	<b>Trial 3</b>	<b>Trial 4</b>	<b>Mean</b>	<b>SD</b>	<b>CV%</b>
1	44932	44063	43474	44364	44208	607.81	1.37
2	49028	50002	49105	49450	49396	443.57	0.90
3	43795	43250	43988	43873	43727	327.41	0.75
4	45079	45163	46221	44680	45286	658.14	1.45
5	44147	46726	46904	47840	46404	1582.17	3.41
6	46832	45115	43899	44541	45097	1258.95	2.79
7	49720	46074	48957	48690	48360	1585.42	3.28
8	46219	44429	47580	47898	46532	1579.54	3.39
<b>Mean</b>	<b>46219.0</b>	<b>45602.8</b>	<b>46266.0</b>	<b>46417.0</b>	<b>46126.2</b>	<b>1005.4</b>	<b>2.17</b>
<b>SD</b>	<b>2192.5</b>	<b>2089.2</b>	<b>2268.6</b>	<b>2261.7</b>	<b>1968.6</b>		<b>1.16</b>
<b>b. Distance Covered Set Workload (metres)</b>							
<b>Cyclist</b>							
1	42780	41588	41296	41068	41683.0	761.7	1.83
2	46701	45997	46366	46626	46422.5	317.9	0.68
3	41274	40667	40960	41426	41081.8	337.8	0.82
4	44101	43224	43538	42812	43418.8	543.4	1.25
5	41738	42638	42198	41833	42101.8	408.8	0.97
6	44139	42857	42937	42660	43148.3	670.7	1.55
7	45281	45308	46441	45808	45709.5	544.6	1.19
8	44043	43153	44006	44757	43989.8	656.2	1.49
<b>Mean</b>	<b>43757.1</b>	<b>43179.0</b>	<b>43467.8</b>	<b>43373.8</b>	<b>43444.4</b>	<b>530.1</b>	<b>1.22</b>
<b>SD</b>	<b>1794.1</b>	<b>1761.3</b>	<b>2085.1</b>	<b>2095.1</b>	<b>1885.0</b>		<b>0.39</b>
<b>c. Distance Covered, 90% Wmax until exhaustion (metres)</b>							
<b>Cyclist</b>							
1	2152	2475	2178	3296	2525.3	534.3	21.2
2	2327	4005	2739	2824	2973.8	720.9	24.2

3	2521	2583	3028	2447	2644.8	261.5	9.9
4	978	1939	2683	1868	1867.0	697.9	37.4
5	2409	4088	4706	6007	4302.5	1494.4	34.8
6	2693	2258	962	1881	1948.5	736.6	37.8
7	4439	766	2516	2882	2650.8	1508.0	56.9
8	2176	1276	3574	3141	2541.8	1026.4	40.4
<b>Mean</b>	<b>2461.9</b>	<b>2423.8</b>	<b>2798.3</b>	<b>3043.3</b>	<b>2681.8</b>	<b>872.5</b>	<b>32.8</b>
<b>SD</b>	<b>953.7</b>	<b>1172.7</b>	<b>1078.8</b>	<b>1310.3</b>	<b>752.1</b>		<b>14.2</b>

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