# LAPORAN AKHIR PROJEK PENYELIDIKAN R & D JANGKA PENDEK

## MAKLUMAT AM Α. Tajuk Projek: Stabilised Oxygen Nutritional Supplement on **Cycling Performance** Tajuk Program: Tarikh Mula: 01 Ogos 1999 Nama Penyelidik Utama: Prof. Rabindarjeet Singh (4813309) (berserta No.K/P) Nama Penyelidik Lain: Prof, Mayda E.T. Larmie (berserta No.K/P) PENCAPAIAN PROJEK B. (Sila tandakan / pada kotak yang bersesuaian dan terangkan secara ringkas di dalam ruang di bawah ini. Sekiranya perlu, sila gunakan kertas yang berasingan). Penemuan asli / peningkatan pengetahuan The use of stabilised oxygen during endurance exercise failed to have any effect on the physiological varibales. Performance . was not enhanced nor was any subjective relief demonstrated. . We therefore can offer no scientific basis for the use of stabilised oxygen in endurance type of athletic activities. For further details, please refer to the attached full report.

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Ph.D	Cheng Chee Keong			
	Mohd Anizu Mohd Nor			
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USM R&D/JP 04-6



Rabindarjeet Singh

IRPA Short-term Project Report

#### Introduction

The basic physiologic response to exercise is an increase in total body oxygen consumption made possible by increase in pulmonary ventilation, cardiac output and oxygen extraction by the tissues. A given work load requires a specific amount of energy. This energy is mainly from aerobic metabolism. Although the energy released in glycolysis is rapid and does not require oxygen, relatively little ATP is resynthesised. It is the aerobic metabolism reaction that takes place in the mitochondria that provides the all important energy sources. Therefore, exercise that is carried out at low to moderate intensity rely solely on aerobic metabolism. As exercise prolongs or becomes heavy, the oxygen supply becomes inadequate or the energy demands outstrip cellular capacity for aerobic resynthesis of ATP. Under conditions of oxygen deficiency, the energy requirement is met by the predominance of anaerobic glycolysis (1).

As participation in international aerobic sports becomes more competitive, athletes and scientists are finding ways and means to enhance aerobic metabolism and delay fatigue. Reviews indicate at there two viewpoints concerning limiting factors for sustained heavy muscular s (2, 3). One view is that the delivery of oxygen to muscles by the cardiovascular systems is limiting and the other is that the metabolic capacity of the muscle to utilise oxygen is limiting. Hence, supplemental oxygen is used by athletes during recovery from vigorous exercise in the belief that it hastens recovery or enhances subsequent performance. It is believed that this procedure significantly enhances the blood's oxygen-carrying capacity and thus facilitates oxygen transport to the exercising muscles. However, in the study of Robbins et al., (4) supplemental oxygen during the recovery period of intermittent maximal activity failed to have any effect on physiological variables. On the other hand breathing hyperoxic gas during submaximal aerobic exercise enhances physical performance (5) and increased endurance time significantly despite, large variations among individuals (6).

Despite the results of studies on supplemental oxygen being equivocal, scientists are continually trying to harness the properties of oxygen for use within the body other than through the breathing process. This has been done through the process of stabilising high concentration of oxygen molecules in dissolved molecular oxygen formulation.

Since scientific evidence for the use of stabilised dissolved molecular oxygen to enhance aerobic performance and endurance has not been addressed, it is therefore proposed that the effect of stabilised dissolved molecular oxygen on physical performance in athletes be investigated. This study examines the effects of stabilised dissolved

molecular oxygen, oxygen enriched water, on physiological responses, and exercise performance of cyclists on a cycle ergometer and to determine its impact on exercise metabolism as well as its effect on thermoregulation and plasma volume changes.

#### **Methods**

Subjects. Seven healthy male avocational cyclists and triathletes participated in this study, and all completed the study. Their mean  $\pm$  SEM age, body weight and height were 28.7  $\pm$  5.8 yrs, 61.5  $\pm$  5.6 kg, and 164.4  $\pm$  1.1 cm respectively. Before starting the experimental trials, the nature and the risks of the experimental procedures were explained and written informed consent was obtained. The study was approved by the ethical committee of Universiti Sains Malaysia.

*Preliminary tests.* A preliminary steady-state exercise and a progressive  $VO_{2max}$  test was administered using a electromagnetically-braked cycle ergometer (Excalibur Sport, Lode). The test consisted of pedaling the ergometer at 50 W for 1 min followed by 16-W increase every minute. The test continued until exhaustion. The mean  $VO_{2max}$  was 50.5  $\pm$  4.4 ml.kg<sup>-1</sup>.min<sup>-1</sup>. Based on the measured  $VO_{2max}$  and  $VO_2$  values from steady-state exercise, an exercise intensity was established which elicited a  $VO_2$  of 50%, 70% and 75% of  $VO_{2max}$ .

To produce homogenous physiological state among subjects, dietary and exercise restrictions were established. Each subject was instructed to record his diet for the 72 h prior to the first experimental session and to eat the same diet preceding the second session. Subjects ate a meal not <8 h nor >10 h before the experimental session and refrained from drinking caffeinated beverage during the described fasting period. In addition, they refrained from training and/or strenuous exercise for 48 h prior to the experimental session.

Experimental design. The subjects cycled until volitional exhaustion on an electromagnetically-braked cycle ergometer at a intensity of 70%  $VO_{2max}$  for the first 90 min and 75%  $VO_{2max}$  thereafter on two different occasions, separated by approximately 1 week. Both trials were performed in the laboratory under similar experimental conditions  $(23.5 \pm 0.09 \, ^{\circ}\text{C})$  and  $61.8 \pm 0.7 \, ^{\circ}\text{C}$  relative humidity). A fan at low speed directed air towards the subjects. On each occasion, the subjects were randomly assigned to consume the oxygen enriched water or water in a series of feedings at every 20 min, which were kept cool at 8  $^{\circ}\text{C}$ , at a rate of 3 ml.kg<sup>-1</sup> body weight. The order of the trials was randomised. A double blind cross-over designed was used.

Endurance Trail. On the day of the experiment, subjects voided their bladder as completely as possible, and nude body weight was then measured (Tanita, Japan, weighing accuracy of  $\pm$  20g). All subjects then had the same standard breakfast,

consisting of 2 slices of bread and a cup of warm water. Then a rectal probe was inserted to a depth of 10 cm beyond the anal sphincter. Four skin electrodes were attached to different parts of the body: chest, biceps, thigh and calf. Subjects were then seated in a room maintained at a temperature of approximately 22°C and remained in a comfortable sitting position for 15 min before a teflon venous catheter was inserted into a forearm vein fitted with a three-way stopcock for blood sampling; this remained in place for the remainder of the study. An initial blood sample was then obtained. All blood samples were obtained without stasis. The catheter was kept patent with a heparin-saline solution (10 IU/ml).

The subject than sat on the cycle ergometer and after sitting for 15 min, a second resting blood sample was obtained and expired gas was measured. Subjects were then asked to warm-up for five minutes by cycling at 50% VO<sub>2max</sub>. Expired gas was collected during the final minute of the warm-up after which the endurance exercise was commenced at the designated intensity. Expired air samples, heart rate, skin and core temperature were taken at intervals of 10 minutes. Subjective ratings of perceived exertion using Borg's scale (7, 8) and fluid sensation (for nausea, fullness and stomach upset) were determined using a fluid sensation scale (9) every 20 minutes. Blood (5 ml) was sampled simultaneously with cardiorespiratory measurements and analysed for [Hb], PCV, glucose, lactate and free fatty acids. Post-exercise nude body weight was obtained after the subjects had towel-dried themselves.

Exhaustion was defined as the time when the subject was no longer able to maintain the designated pedal rate of 60 rpm. Prior to the tests the subjects were instructed as to the importance of continuing exercise until completed exhaustion. No verbal or other encouragement was used during the experiment. When the subjects approached exnaustion measurements were resumed irrespective of the above time table.

Techniques. For the preliminary and experimental trials, the subjects were fitted with a head gear which supported a one-way non-rebreathing mouth piece (Vacummed 2700B). VO<sub>2</sub> and related variables were measured using computerised gas analysis system, (SensorMedics 2900). The oxygen content was analysed with a paramagnetic oxygen analyser and carbon dioxide with an infra-red carbon dioxide analyser. The analysers were calibrated daily using nitrogen based calibration gases. Core and skin temperatures were recorded by a temperature monitor (Libra Medical ET 300R). Heart rate was obtained from an electronic pulse tester (Sport Tester PE 300, Polar Electro KY, Kempele, Finland). Mean skin temperature (Tsk) was derived by using the formula of Ramanathan (10).

Samples for glucose and free fatty acids centrifuged and frozen (-20° C) and later analysed. Plasama glucose and free fatty acids concentrations was measured

spectrophotometrically by an enzymatic colorimetric method (Boehringer and Wako chemical Industries, Japan respectively). Plasma lactate was measured by means of an enzymatic method (lactate analyser model 2900, Yellow Springs Instruments, Ohio, USA). Hemoglobin was measured by cyanmethemoglobin method and hematocrit was determined in duplicate after microcentrifugation. The percent change in plasma volume (%ΔPV) was calculated according to Beaumont et al., (11). All analysis were run in duplicate and both experiments for one subject were analysed on the same day to obviate the day-to-day variability of the procedures.

Statistics. Results are expressed as the mean ± SEM. Between-group differences from the biochemical assays and cardiorespiratory measures were analysed using one-way analysis of variance (ANOVA). The Statistical Package for Social Sciences (SPSS) programme was used for statistical analysis. P values of less than 0.05 were taken to indicate statistical significance.

#### Results

Time to exhaustion averaged 101.7  $\pm$  6.0 min (range 81.7-123.9) with oxygen enriched water and 98.5  $\pm$  7.5 min (range 72.3-133.3) with water. The endurance time (Fig 1.) was 2.4% longer with oxygen enriched water. The VO<sub>2</sub> averaged 71% of VO<sub>2max</sub> in both trials after 10 min of exercise and continued to increase slowly over the rest of the exercise period to 79% at exhaustion (Fig 2). There were no significant differences in VO<sub>2</sub> between oxygen enriched water and water, nor were there in RQ which stabilised at a mean of 0.94 once steady-rate was attained. The heart rate were similar in both trials but

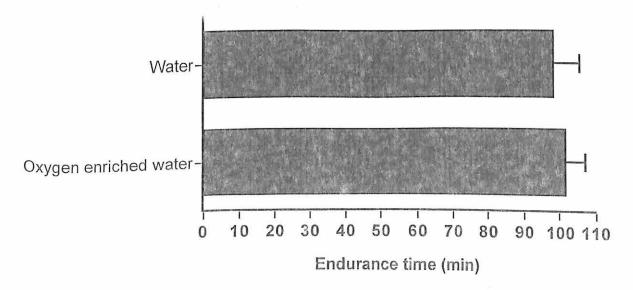


Fig. 1. Time to exhaustion during cycle exercise at 71% of maximal oxygen uptake with oxygen enriched water and water (mean±SEM; n=7)

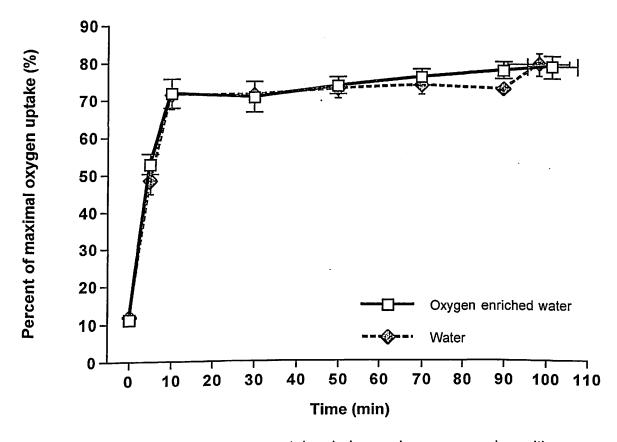


Fig 2. Percent of maximal oxygen uptake during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

at exhaustion it was 4 beats min<sup>-1</sup> lower with oxygen enriched water, but the difference was non-significant (Fig 3).

Plasma glucose decreased throughout the endurance exercise in both trials and stabilised at about 4.6 mmol\*L-1 with oxygen enriched water which was higher than with the water trial but was not significantly different (Fig 4). However, at 20 min, plasma glucose with water trial was significantly lower (p<0.05) than with oxygen enriched water. Plasma lactate increased from an average value of 1.8 mmol\*L-1 and continued to drift upwards during endurance exercise to a level about 4 mmol\*L-1 at exhaustion in both trials (Fig 5). Hemoglobin concentration increased by approximately 9% during the endurance exercise in both trials (Fig 6).

PCV was lower at rest with oxygen enriched water but was not significantly different from the water trial. The values increased by approximately 4% during endurance exercise but retained the difference between oxygen enriched water and water (Fig 7). Plasma volume declined significantly during the endurance exercise in both trials to  $-7.21\pm1.6\%$  and  $-8.7\pm2.2\%$  at exhaustion with oxygen enriched water and water respectively (Fig 8).

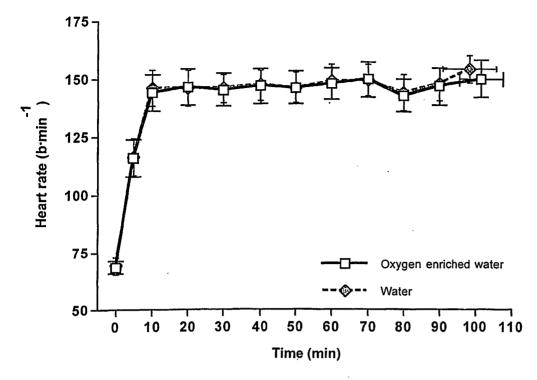


Fig 3. Heart rate during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

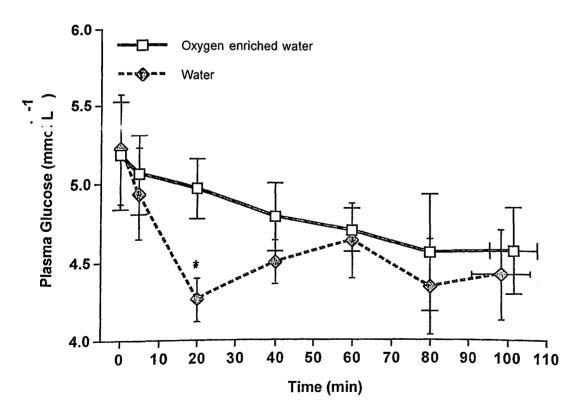


Fig 4. Plasma glucose during endurance exercise with oxygen enriched water and water (mean±SEM; n=7). \* significantly different at p<0.05.

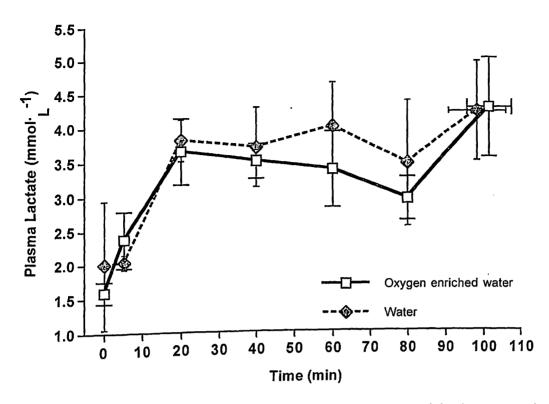


Fig 5. Plasma lactate during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

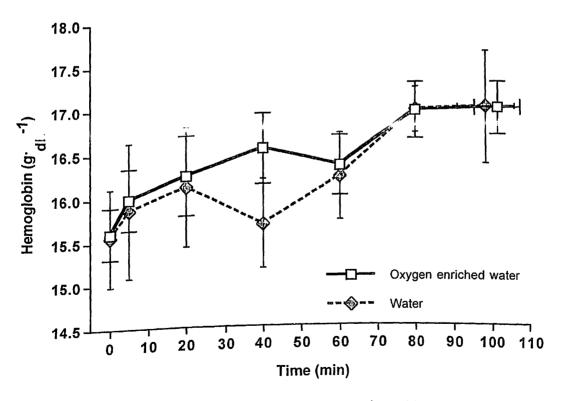


Fig 6. Hemoglobin concentration during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

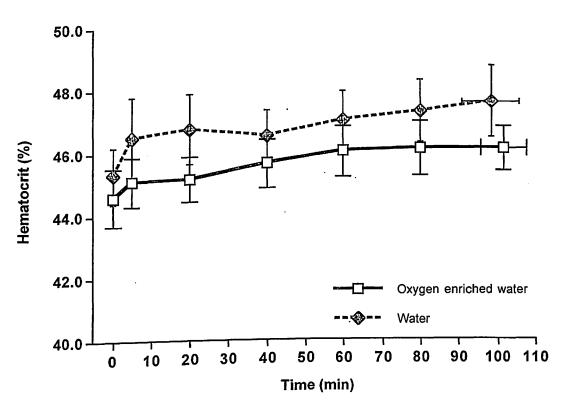
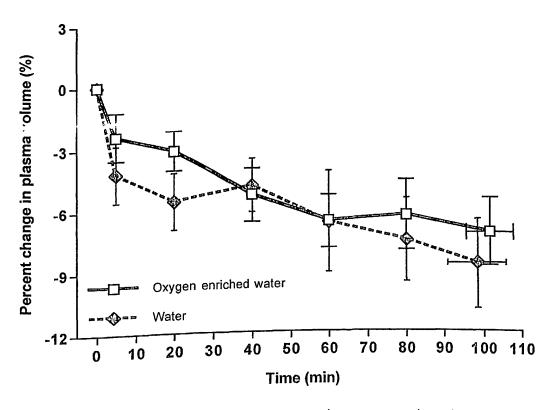


Fig 7. Hematocrit during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).



**Fig 7.** Percent change in plasma volume during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

Core temperature increased from 36.8°C at rest to about 38°C at exhaustion in both trials with no differences between trials (Fig 8). However, mean skin temperature decreased from a value of 31.5°C at rest to approximately 29°C at exhaustion, again with no differences between trials (Fig 8).

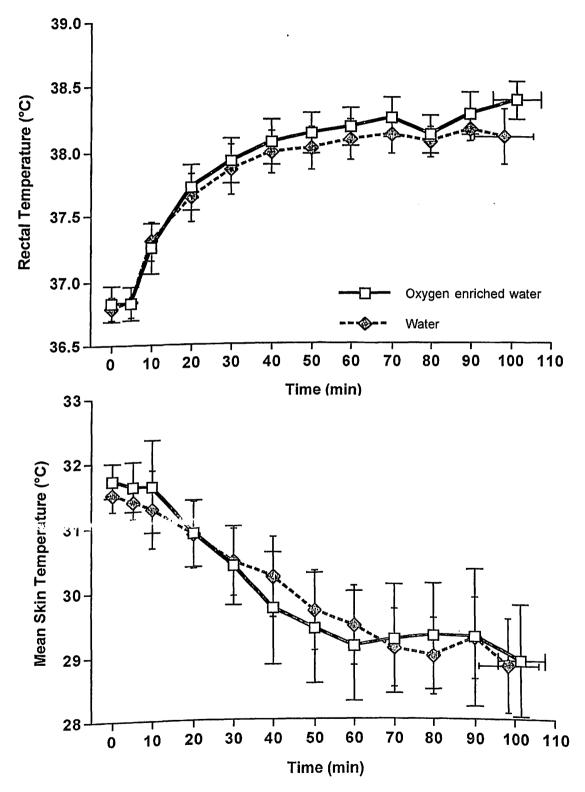


Fig 8. Rectal and mean skin temperatures during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

The total volume of fluid intake, decrease in body weight corrected for the fluid ingested during exercise and the percent change in body weight were similar with oxygen enriched water and water trials (Table 1). The perceived rate of exertion and fluid sensation for thirst, nausea, fullness and stomach upset from rest to exhaustion were similar during the endurance exercise with oxygen enriched water and water (Table 2).

**Table 1.** Total fluid intake, change in body weight and percent change in body weight during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

Fluid type	Total fluid intake (ml)	Change in body weight (kg)	Percent change in body weight (%)
Oxygen enriched water	987.6±130.0	1.43±0.29	2.31±0.40
Water	931.7±169.7	1.69±0.16	2.77±0.18

**Table 2.** Perceived rate of exertion and fluid sensation scale for thirst, nausea, fullness and stomach upset during endurance exercise with oxygen enriched water and water (mean±SEM; n=7).

Fluid type	Min 20	Min 40	Min 60	Min 80	Exhaustion
Perceived rate of exertion (PRE)					
Oxygen enriched water	10.00±0.79	11.71±0.89	13.00±1.11	13.20±1.83	15.14±2.79
Water Water Thirst (1=not thirsty)	10.00±0.62 ; 5 extremely th	11.71±0.81 nirsty)	12.29±0.89	13.50±1.02	16.00±1.23
Oxygen enriched water	1.29±0.18	1.71±0.29	1.71±0.36	1.80±0.58	2.40±0.75
Water Nausea (1=no naus	1.29±0.18 ea; 5=extreme	1.57±0.30 ly nausea)	1.86±0.40	1.50±0.34	2.29±0.52
Oxygen enriched water	1.14±0.14	1.14±0.14	1.29±0.29	1.60±0.60	1.67±1.63
Water Fullness (1=not full;	1.00±0.00 5=extremely for	1.00±0.00 (III)	1.00±0.00	1.29±0.29	1.29±0.29
Oxygen enriched	1.00±0.00	1.14±0.14	1.29±0.29	1.60±0.60	2.00±0.68
water Water Stomach upset (1=r	1.29±0.29 no upset; 5-exti	1.29±0.29 remely upset)	1.43±0.30	1.17±0.17	1.29±0.29
Oxygen enriched	1.00±0.00	1.43±0.30	1.57±0.37	1.60±0.60	2.00±0.68
water Water	1.00±0.00	1.29±0.29	1.43±0.43	1.00±0.00	1.43±0.43

#### Discussion

In this study we have shown that acute ingestion of oxygen enriched water has no discernible effect on endurance cycle at 70% of maximal oxygen uptake. We were unable to demonstrate that acute ingestion of oxygen enriched water has any significant influence on the metabolic responses during endurance exercise. In addition, oxygen enriched

water did not alter the perception of the magnitude of exertion as determined by the Borg scale. The subject were unable to discern when oxygen enriched water was in used.

Most studies on the effect of hyperoxia on exercise performance have addressed short-term exercise capacity, i.e. exercise tolerance at or above the rate of maximal oxygen uptake (12, 13, 14), however, the use of oxygen gas during exertion also appears to be beneficial in prolonging endurance and easing dyspnea (5, 14, 15). To our knowledge there is little evidence on the use of oxygen enriched water during short or long term exercise.

Maximal heart rate is apparently not affected by hyperoxia (12, 13, 16-18) but during submaximal exercise, there appears to be a reduction in heart rate with elevated PO<sub>2</sub> (19-21). This reduction in heart rate, although small appears to be real; the mechanism is unclear at this time, although there is some speculation that the response may be mediated by the peripheral chemoreceptors (19). Our data, using oxygen enriched water showed similar heart rate responses with water during endurance exercise. The time to exhaustion with oxygen enriched water (101.7±6.0 min) tended to be longer compared with water (98.5±7.5 min) but did not reach statistical significance (p>0.05). Whether this difference represents inherent variability in this physiologic measure or a real effect of oxygen enriched water on endurance performance is a question a larger study could address. It could also be a reflection of individual variation. The average 2.4±6.4% increase covered a range from -15% to +26%, was also noted in the study of Plet et al (6) using hyperoxia.

Depletion of muscle glycogen has been proposed as a cause of fatigue in prolonged, submaximal exercise where in some studies have reported lower RQ values with hyperoxia as indication of a reduced carbohydrate utilisation (13, 22). In the present study, RQ values were the same in oxygen enriched water and water. RQ measurements are however, in general difficult to interpret and perhaps of limited value as indices of substrate utilisation. Firstly, the VO2 measurements are technically demanding and even small errors may lead to quite gross overestimates. Secondly, differences in breathing pattern between oxygen enriched water and water may lead to variations in VCO2, which have nothing to do with substrate utilisation although the R value has changed. The safest thing to conclude may therefore be that the fact that despite builtin pitfalls, we did not see any differences in RQ or VO2 between oxygen enriched water and water trials provided an extra argument that the slight longer time to exhaustion was not caused by differences in substrate utilisation (notably muscle glycogen depletion). The observation of similar plasma glucose in oxygen enriched water and water was also in accordance with this view (fig 4).

One of the more important observations related to O<sub>2</sub> inhalation during exercise is the effect on lactic acid metabolism where it is well accepted that for submaximal exercise, blood lactate levels are reduced with hyperoxia (23). It has been assumed that the reduction of lactate levels during hyperoxia is a result of alleviating the anaerobic conditions in working muscles. However, lactate production by cell without mitochondria, red blood cells, is also depressed by increased PO<sub>2</sub> (24), which suggests that the effect of hyperoxia on blood lactate levels during exercise may be the result of something other than tissue hyperoxia. In our study, plasma lactate levels were lower although not significantly with oxygen enriched water but were similar at exhaustion.

In summary, the use of oxygen enriched water during endurance exercise failed to have any effect on the physiological variables. Performance was not enhanced nor was any subjective relief demonstrated. We therefore can offer no scientific basis for the use of oxygen enriched water in endurance type of athletic activities.

### Acknowledgement

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