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University  
of Glasgow

**The effects of New Zealand Blackcurrant on critical velocity and speed  
tolerable-duration relationship during running**

Written project report submitted in fulfilment of the requirements for the Degree of MRes in  
Sports Science of the Institute of Cardiovascular and Medical Sciences

**Matric Number:**

**Date of Submission: 16th October 2018**

**Supervisor 1: Dr. Ole J. Kemi**

**Supervisor 2: Mr. Nairn Scobie**

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### List of Abbreviations

ANOVA	Analysis of Variance
AT	Anaerobic Threshold
a-v O <sub>2</sub>	Arterio-Venous Oxygen Difference
AWC	Anaerobic Work Capacity
BC	Blackcurrant
BF	Body Fat
BS	Baseline
BMI	Body Mass Index
CF	Critical Force
CHO	Carbohydrate
CP	Critical Power
CS	Critical Speed
CT	Critical Threshold
CV	Critical Velocity
D'	Fixed work above CV (running)
DOMS	Delayed Onset of Muscle Soreness
EE	Energy Expenditure
FW	Fat Weight
GLM	General Linear Model
HIIT	High Intensity Interval Training
HR	Heart Rate
HR max	Maximal Heart Rate
IET	Incremental Exercise Test
IL-17	Interleukin-17
La	Lactate
LT	Lactate Threshold
LW	Lean Weight

MAP	Mean Arterial Pressure
NZBC	New Zealand Blackcurrant
PCr	Phosphocreatine
PLA	Placebo
PO	Power Output
RE	Running Economy
REE	Resting Energy Expenditure
ROS	Reactive Oxygen Species
RPE	Rate of Perceived Exertion
SD	Standard Deviation
SV	Stroke Volume
t	Time
TT	Time Trial
TTE	Time to Exhaustion
V	Velocity
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_2 \text{ max}$	Maximal Oxygen Uptake
W'	Fixed work above CV (cycling)
W	Weight
WR	Work Rate

## 0.0 Abstract

**Background:** Supplement intake is used to support training practice and performance enhancement. Performance enhancing techniques include anything from a training plan and a good nutrition to supplement consumption and legal substances. Research is now focused on the functional food ingredients of these ergogenic aids or also called superfoods. Blackcurrant (BC) is considered as one of the superfoods and there is research going on in the literature examining its effects on both health and performance. In order to maximize training at severe intensity exercise domain, the currently new concept of Critical Velocity (CV) could be used from which greater number of benefits may occur.

**Aim:** (i) To test the reproducibility of CV and speed-tolerable duration in runners and non-runners (controls) and (ii) examine the effects of New Zealand Blackcurrant (NZBC) based on these two parameters.

**Methods:** In the first part of the study, eight (N=8) runners and nine (N=9) controls volunteered for participation and performed two series of exhaustive tests. For CV and D' determination, tolerable-duration ( $V-t$ ) and *Velocity – 1/time* (inverse of time) relationships were plotted. In part 2, the Blackcurrant (BC) supplementation intervention study, seven (N=7) young, healthy, recreationally active males participated. Testing comprised of resting measurements (ventilation hood, Bodpod, and resting lactate), Incremental Exercise Test (IET) and four Constant Load Tests which were randomized over two phases either on BC or Placebo (PLA) supplementation.

**Results and Conclusions:** The reproducibility tests showed that there was no statistical difference for Maximal Oxygen Uptake ( $\dot{V}O_2$  max) and Maximal Heart Rate (HR max) during the Constant Load Trials and IET for both runners and controls. This confirms that exhaustion was always reached in all tests. Mean CV as well as distance covered (D') were not different for the runners between the test and retest. However, there was a difference in these parameters between the test and retest for the controls. This supports validation of the hypothesis that CV and D' are reproducible for the runners and not for the non-runners.

For the BC supplementation study, mean values for CV and D' showed no discernible difference between BC and PLA subjects. Subjects under BC supplementation produced approximately the same CV value when compared to the ones under PLA. This implies that BC has little effect on CV. However, when looking at individual results, 60% of the subjects (4 out of 7) showed an increase in D' under the effect of BC, even up to 41%. Two subjects showed a decrease of the order of 10%. This implies that BC enhancement may improve D' but more specific studies are proposed to decipher the factors. The above are supported by measurements of Heart Rate (HR), Oxygen uptake ( $\dot{V}O_2$ ), Energy Expenditure (EE), Fat oxidation (Fat), Carbohydrate oxidation (CHO) and Running Economy (RE) all of which showed small or no effect with intervention.

## **1.0 Introduction**

### **1.1 Overview**

#### **1.1.1 Overview/Role of exercise**

Exercise provides important health and performance benefits, and can also be used against disease, for prevention and treatment. The terms Physical Activity, Exercise and Physical fitness are terms that have previously been used interchangeably but they describe different concepts. Physical activity is by definition ‘‘any bodily movement that is carried out by the skeletal muscles resulting in energy expenditure (EE)’’ for instance sports, conditioning, household or other activities. Exercise is a subset of physical activity which is planned, structured and repetitive. Exercise has a final or an intermediate aim to improve or maintain the physical fitness. A set of attributes which are either health- or skill-related describe the physical fitness (Caspersen et al., 1985). According to epidemiological data, being physically active contributes to significant health benefits (Bassuk, 2005). Exercise training has demonstrated a decrease in risk factors for cardiovascular disease, metabolic syndrome, type 2 diabetes, cancer, stroke, high blood pressure, stress, depression and anxiety. Exercise training enhances the immune and respiratory system and also reduces the risk of musculoskeletal injury with conditioning, warm up, and stretching.

#### **1.1.2 Role of exercise intensity**

As a result of training intervention, an improvement in metabolic and cardiovascular function and also an increase in  $\dot{V}O_2$  max have been shown (Blair, 1996), as well as delayed exercise intolerance and performance enhancement. The main key for these changes and improvements is considered to be the intensity of exercise. Various forms of training have been acknowledged alternatives to increase the adherence to the training programmes, with the objective to decrease the time spent exercising while augmenting the exercise intensity. High-Intensity Interval Training (HIIT) is described as short periods of exercise at high intensity (>80-85% peak oxygen uptake), alternated with periods of active or passive rest. HIIT is considered as a beneficial option compared to moderate-intensity continuous training to enhance time efficiency. Continuous high-intensity exercise has suggested greater benefits than moderate-intensity according to Swain and Franklin (2002). Time-trial performance and endurance capacity, muscle oxidative enzyme activity, aerobic capacity ( $\dot{V}O_2$  max), insulin sensitivity, vascular function and blood pressure were all improved with the HIIT in a time-efficient way (Gibala et al., 2006). In order to improve health, fitness and performance, an appropriate program should be designed with daily exercise. From an athlete’s perspective, training is important to stress the body in order to cause the adaptations and also inferring an increase in physiological can lead to increase in performance (Sawka et al., 2000). However, based on the genetic responses at different intensities, these adaptations may be caused for both health and performance, where the recovery and training status, the regularity, duration, intensity and type of exercise are also very critical (Barisic et al., 2011). Genetic factors have strong effects on performance measures such as explosive strength, speed of limb movement, running speed, reaction time, balance and flexibility. Characteristics including blood lipid and coagulation profile as well as mitochondrial activity and blood pressure are also influenced by an athlete’s genetic profile. Nonetheless, exercise response heterogeneity exists and is

influenced by both endogenous (age, sex, race) and exogenous factors (mode, intensity, duration, time of day, nutritional status) (Sparks, 2017).

Currently, in order to develop a training program, different methods can be used for intensity prescription based around a percentage such as  $\dot{V}O_2$  max, maximal Heart Rate (HR max), whereas for blood lactate (BLa) and rate of perceived exhaustion (RPE) the subject needs to exercise in between the heavy (blood lactate is increased above resting values but is stable over time) and severe (blood lactate is elevated continuously throughout constant-intensity) exercise domain (Smith and Jones, 2001). To establish optimal training, a more individualized method has been considered, known as the Critical Threshold (CT), so that aspects of performance ( $\dot{V}O_2$  max and exercise capacity) or performance itself can be improved. Health implications including increased quality of life and reduced risk of all-cause mortality and cardiovascular and metabolic disease may also be caused by CV (Moholdt et al., 2012).

### 1.1.3 Speed-tolerable duration relationship and Critical Velocity for intensity demarcation

Greater benefits are provided from continuous high-intensity exercise for cardiorespiratory fitness, where the risk factors of cardiovascular disease are also reduced compared to low-to moderate intensity exercise. The intensity of exercise is an essential factor in order to determine the benefits of exercise training. Different methods that use various parameters for exercise tolerance definition include  $\dot{V}O_2$  max, HR max, LT and CV.

The  $\dot{V}O_2$  max is defined as “the maximal rate at which oxygen is utilized by the body during exercise” and is the most common way of aerobic fitness measurement. However, it is a limiting factor when considering endurance performance, as no athlete can perform above 100%  $\dot{V}O_2$  max (Bassett and Howley, 2000). The LT describes the increase in the blood lactate concentration in association with exercise intensity and it can also be called as “the boundary between moderate and heavy intensity exercise” (Whipp, 2002). According to Monod and Scherrer (1965), the Critical Power (CP) is “the maximum rate of work, that the muscle can sustain for a very long time without reaching fatigue” and can also be considered as “the upper boundary of the heavy intensity domain and the lower boundary of the severe exercise intensity domain” (Jones et al., 2007). CP is a more individualized method that may improve performance or aspects of it and establish optimal training.

The two components that define the speed-tolerable duration relationship is the asymptote of the power or velocity, known as CP or CV and the constant  $W'$  or  $D'$  which is given by the formula  $W' = t * (P - CP)$  where  $t$  is time and  $P$  is power, or  $D' = t * (V - CV)$  where  $V$  is velocity. CV is the work rate which theoretically could be sustained indefinitely, or for a very long time without the onset of fatigue.  $W'$  or  $D'$  is the fixed quantity of work or energy source which is fuelling the particular exercise above CV or CP and can be depleted at different rates. It is also the amount of work that is performed anaerobically before  $\dot{V}O_2$  max is reached.

One of the uses of the CV is for exercise intensity prescription for interval training programs. The tolerable duration of exercise is above CV; therefore, the V-t relationship may define the prescription. However, the prescription and measurement of exercise intensities can cause certain difficulties. The biggest concern is that everyone is different, and every individual has their own exercise requirements. The % $\dot{V}O_2$  max and %HR max for example, may be used for exercise prescription, in order to normalize values for each individual as everyone has

different values for  $\dot{V}O_2$  max and HR max. These are two common ways to prescribe exercise intensity. However, when considering elite level athletes and individuals want to gain the most benefit from training, this percentile normalization is not as effective due to the fact that slight changes in percentage can cause different responses depending on the fitness of the individual. The other problem with the % $\dot{V}O_2$  max and %HR max is that these percentages do not ensure exercise in the high or heavy intensity domain, compare to the CV, when the individual is working above the CV value then the heavy intensity is guaranteed.

Another issue is the measurement and testing of each parameter. A great problem of the CV measurement is the unknown reproducibility. It is also possible that there is a difference in the reproducibility of the speed-tolerable duration relationship in runners. If that is the case, then it is possible to improve it with runners that are accustomed to running on a treadmill and are more able to pace themselves over longer periods of time. Comparing to runners, the non-runners may be good at running but not as good to pace themselves at the time trials, that are a requirement to establish the CV and tolerable duration relationship.

Therefore, four tests need to be carried out in order to plot the relationship, which is not linear but more like an exponential decay. For this, the coefficient of variation between the four tests is assessed, and then the reproducibility of the CV is also assessed which is unknown. It is important to measure the reproducibility of the CV concept so that athletes and coaches can use it to establish optimal training and performance. The reproducibility is required, and it is beneficial to develop and accept the CV concept.

#### 1.1.4 Ergogenic aids

For many years, performance enhancement has been a major concern in sport. Performance enhancement is either legal (vitamins or other substances) or illegal (drugs or other banned substances). In order to support training practice and enhance performance, supplement intake has become very common. For both health and performance enhancement, scientific research is now focused on the functional food ingredients of these ergogenic aids (Shipp and Abdel-Aal, 2010). Ergogenic aids by definition are mechanical, pharmacological, nutritional, physiological and psychological tools that are used by athletes to improve their energy, performance and recovery. Commonly used ergogenic aids are for example amino acids, caffeine, creatine and protein powders (Marcus, 2014). Ergogenic aids can be pills, drinks, powders, bars and gels that may increase an athlete's endurance, strength, recovery, energy levels or body composition and can result in performance enhancement. Usage of dietary supplements and ergogenic aids in exercise performance are considered as an evolving and productive market. Up to date, very few ergogenic aids showed significant improvement in performance.

#### 1.1.5 Blackcurrant extract

Fruit and vegetables are considered as multi-ingredient foods which contain anthocyanin. Due to its antioxidant and anti-inflammatory activity, anthocyanin is often linked to health benefits and is also responsible for the colours of the fruits and vegetables. Particularly, New Zealand Blackcurrant (NZBC) contains high anthocyanin content probably because of the growing and environmental conditions (Cook, 2018). Blackcurrant (BC) has also been considered as an ergogenic aid, as there is research going on in the literature recently, examining its effects on health, performance and recovery. BC health and performance

benefits may have implications for nutritional strategies to be used by athletes for training, endurance exercise, high-intensity intermittent exercise and recovery.

#### 1.1.6 Aims of study

The first aim of this study was to test whether CV is reproducible over two series of exhaustive tests on controls (non-runners) and runners. The aim was to establish the CV and speed-tolerable duration in each subject with the use of four-time trials and then re-establish it with another set of the same time trials. We would then assess whether a different CV and speed-tolerable duration relationship was produced, between controls (non-experienced to running) and runners (experienced to running). This aim will be explored in chapter 3.

The second aim was to examine the effects of NZBC on CV and speed-tolerable duration relationship during running and resting metabolism. Specifically, we aimed to establish the CV and speed tolerable duration in each subject with the use of four-time trials and then try to re-establish it with the same time trials, in order to test whether different CV was resulted between intervention (BC supplementation) and non-intervention (PLA supplementation). This aim will be explored in chapter 4.



## 2.0 Methods

### 2.1 Participants

All subjects who volunteered to take part in this study were recreationally active males, aged 18-45 years old, who were free from illness or any other medical condition. Participants should be non-smokers and be refrained from any nutritional supplements (proteins, anabolic steroids, drugs or other forms of doping). Height and weight were recorded at the screening session by using a wall stadiometer and a calibrated balance. Subjects were recruited from the University of Glasgow (Athletics Club, Sport and Recreation, Sports Science Department) and also from the Crossfit gym Glasgow.

At least 11 visits to the lab were required over the period of 4 weeks per subject. Procedures involved a maximum of 3 tests a week with 48 hours rest between each trial at the same time of day ( $\pm 1$  hour). Instructions were given to refrain from strenuous training or exercise and alcohol 24 hours before testing, caffeine for 4 hours and food within 2 hours for all testing procedures, and this was consistent for all experimental tests. Wash out period of 14 days only took place on the second part of the study which involve an intervention (BC supplementation).

Participants did not receive financial incentive or compensation for participating in this study.

### 2.2 Ethics

All procedures were approved by the University of Glasgow, MVLS College Ethical Committee for non-clinical research. All the experimental procedures, risks and benefits were explained in the written informed consent along with the screening session. In order for the subjects to get cleared to participate in the study they also had to complete a pre-test health questionnaire. Subjects had to sign the written consent prior to participation and they also had the right to withdraw from the study at any stage without question.

### 2.3 Design

Participants visited the laboratory at least 11 times where they were required to perform exhaustive treadmill tests,  $\dot{V}O_2$  max and  $\dot{V}max$  (the speed at which  $\dot{V}O_2$  max is achieved). The Constant Speed Tests were performed at different percentages of  $\dot{V}max$ . No more than 3 experimental tests were performed in any given week.

### 2.4 Calibration

Prior to each test, the gas analyser (Servopro 4100, Servomex, Texas) was calibrated where known concentrations and room air were used (Bergstrom, 2014). Room and barometric pressure were also recorded before and after each trial. Calibration of the analysers prior to each test was essential, according to manufacturer's guidelines. Usage of precision analysed gases spanned the physiological range of inspired and expired gas concentrations. Gas

mixtures were re-sampled after the test so that the stability relation to the initial gas concentration would be confirmed. Lactate analyser was also calibrated before and after the tests with an 8 mm/l standard.

### 2.5 Equipment and Measurements

For all exercise tests, a motor driven programmable treadmill (PPS Med, Woodway, Weil am Rhein, Germany) was used at a 1% gradient. The 1% gradient was used for the lack of air resistance consideration with indoor treadmill exercise so that the energetic cost of the treadmill exercise with that of outdoor running could be matched (M. Jones and Jonathan H. Doust, 1996). A nose clip and a mouthpiece were worn at all tests. A large 2-way non-rebreathing valve (2700 series, Hans Rudolph, Shawnee, KS, USA) was connected with a mouthpiece to collect respired gas (via a 1.5 m length of 3.5 cm diameter tubing) in a Douglas bag. To calculate the gas exchange variables (especially  $\dot{V}O_2$ ), expired gas concentrations collection with the analyzers (Paramagnetic (O<sub>2</sub>) and Infrared (CO<sub>2</sub>) analysers; Servopro 4100 gas analyser, Servomex, Crowborough, UK) and gas volume (Dry gas meter; Harvard Apparatus, Edenbridge, UK) were required. For HR determination, a short-range telemetry HR monitor (S610i, Polar Electro Oy, Kempele, Finland) was used in all tests. HR was recorded every 5 seconds on the SRM Powercontrol III (SRM training system) data logger. When the HR was recorded on the data logger, then the data were later downloaded to a computer via an interface lead for further analysis.

### 2.6 Incremental Exercise Test (IET)

The IET involved a warm-up of 6 minutes at 8 km/hr followed by a speed increase of 1 km/hr automatically every minute until the subject would reach exhaustion. The treadmill was set at 1% gradient to match the energetic costs of outdoor running and to replicate the absence of air resistance (Jones et al., 1996).

Collection of expired air samples and HR was used as verification criteria of maximal effort for the Constant Load Tests. Between 8-15 minutes subjects achieved maximal effort. Verification criteria were HR max (220-age  $\pm$  10 bpm), respiratory exchange ratio (RER)  $\geq$  1.10, volitional exhaustion and  $\dot{V}O_2$  peak l/min<sup>-1</sup> within 200 ml of maximal value (Gaesser and Pool, 1996).

Douglas bags were used for expired air samples collection continuously every minute until exercise intolerance. To verify the subject's maximal effort, the final two bags were analysed. At exercise intolerance, the participant straddled the treadmill, despite strong verbal encouragement. Recovery was then followed, to ensure the subject's safety, where the speed was decreased to 4 km/hr for approximately 5 minutes. Active recovery would help the participants to recover their blood lactate level quicker than immediately resting as well as return the HR to its normal value. It also gets the blood moving and helps reduce residual fatigue in the muscle.

### 2.7 Constant Load Tests

Based on the maximal speed that the subject achieved at the incremental ramp test, the Constant Load Tests were individualised to each subject. These tests were designed in such way in order to elicit exhaustion within 3 to 15 minutes (Moritani et al., 1981).

The tests commenced with a 5.5 km/hr brisk walk for 6 minutes and then the treadmill would accelerate to the pre-programmed speed for the particular trial. The trial's duration was recorded by two stopwatches in case of error or malfunction, until the participant would reach exhaustion.

Serial expired gas samples were collected in Douglas bags (60 s collection) when the subject was considered to be approximately 2 minutes from exercise intolerance, so that the  $\dot{V}O_2$  peak could be established. The  $\dot{V}O_2$  peak was confirmed as  $\dot{V}O_2$  max when there was no difference in the  $\dot{V}O_2$  peak attained with increases in constant-speed tests ( $\pm$  200 ml). Four Constant Load Tests were required for every participant at each phase, at different velocities so that the CV and D' were attained whilst conforming to Standard Error of Estimates (SEE) before repeating the tests in the second phase in a randomised order. At least 8 Constant Speed Tests were completed with  $\geq$ 24 hours difference for each trial.

### 3.0 Reproducibility of Critical Velocity and Speed-Tolerable Duration

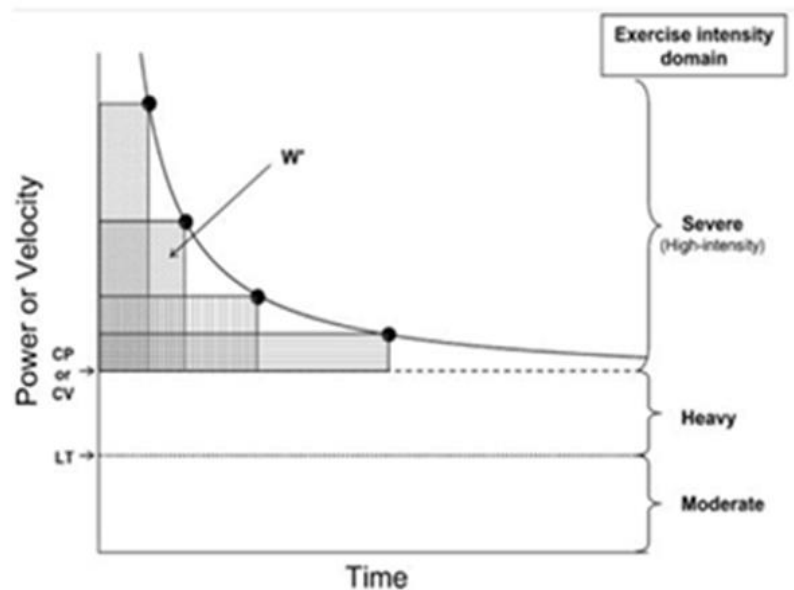
#### 3.1 Introduction

When the exercise intensity is set by using  $\dot{V}O_2$  max and HR max percentages, two main parameters are ignored, which define the exercise tolerance. These parameters are the lactate threshold (LT) and critical power (CP) or Critical velocity (CV). LT is defined as the point where  $La^-$  removal is exceeded by  $La^-$  production, resulting in the accumulation of metabolic by-products. The boundary between moderate and high intensity exercise is demarcated by the LT.  $La^-$  is accumulating when this boundary is crossed and exercise is performed in the heavy or severe intensity domains above LT, where acidosis occurs (see also Figure 1) (Whipp, 2006).

Critical Velocity, by definition, is described as “the threshold intensity above which exercise of sufficient duration will lead to attainment of  $\dot{V}O_2$  max” (Hill and Ferguson, 1999). Vanhatalo et al., (2011) described this concept as the boundary between non-steady state and steady state exercise intensity domains (heavy and severe) which can represent a better index of endurance performance than the current thresholds. The basis of CV or CP, in running and cycle ergometer exercise, is characterized by a hyperbolic model, which describe the relationship between power and time to exhaustion (TTE) (see also figure 1) (Moritani et al., 1981). TTE is a function of anaerobic work capacity (AWC) and CV and is given in the formula  $TTE = AWC*(V-CV)^{-1}$  (Moritani et al., 1981). AWC is considered as the amount of work performed without the support of aerobic metabolism and is related to certain indicators of anaerobic capacity such as work in intermittent high-intensity exercise, work in 30 seconds and maximal  $O_2$  deficit (Hill, 1993). Aerobic fitness indices including LT or  $\dot{V}O_2$  max are related to CV (Moritani et al., 1981). The physiological basis for CV or CP is not yet known.  $\dot{V}O_2$  increases rapidly and then levels off, at the beginning of exercise below LT (Whipp and Wasserman 1972). A second slow component follows above the LT and after the rapid initial increase in  $\dot{V}O_2$ , resulting in a delayed steady state (Henson et al., 1989). The slow component of the  $\dot{V}O_2$  response will lead  $\dot{V}O_2$  to  $\dot{V}O_2$  max instead of a delayed steady state, when the exercise is performed above CP. During constant power exercise,  $\dot{V}O_2$  max can be achieved at a range of intensities (Gaesser and Poole, 1996). As said above, the hyperbolic model describes the relationship between intensity and TTE. The same occurs with intensity and time to achieve  $\dot{V}O_2$  max (TTmax) and is given in the form  $TTmax = AWC'*(V-CV')^{-1}$ . CV' is defined as the highest submaximal exercise intensity that can be sustained without reaching  $\dot{V}O_2$  max. The amount of work performed anaerobically before  $\dot{V}O_2$  max is elicited is called AWC' (Hill and Ferguson, 1999).

Unlike other aspects of performance such as BLa, HRmax and  $\dot{V}O_2$  max, the speed-tolerable duration relationship is based on performance measurement rather than physiological index (Fitts, 1994). The relationship can also be transferred into a linear model where CV is the slope of the regression of speed on time. Critical Velocity (CV) or Critical Speed (CS) would then be considered as the maximum rate that could theoretically be sustained indefinitely or more realistically for a very long time without fatigue (Monod and Scherrer, 1965). The formula which describes the hyperbolic Velocity-tolerable duration relationship equation is  $V=(D'/t)+CV$ . V is the velocity at which the treadmill is set in at km/hr. The desired tolerable

duration is described by the letter  $t$  in seconds. CV is the critical velocity of the participant in km/hr and  $D'$  is the amount of work that is performed anaerobically before  $\dot{V}O_2$  max is achieved.



*Figure 1: Shows the hyperbolic model which describes the power or velocity over time to exhaustion. Illustrates where Critical Velocity (CV) occurs in relation to Lactate Threshold (LT) depending on the exercise intensity whether it is moderate, heavy or severe. It is expected that CV will be lower for lower intensities, if the LT of an individual is low.*

### 3.1.1 Critical Velocity (CV) and $D'$

The time achieved during continuous exhaustive bouts of running at different velocities is required in order to calculate CV (Monod and Scherrer, 1965). In order to predict the TTE at a velocity which exceeds the CV point, the resultant CV can be used from the mathematical modelling which is described above. Overestimation in practice may occur regarding the duration that CV can be maintained, where runners may sustain running exercise between 15-30 minutes (Vanhatalo et al., 2011). Therefore, CV is considered as the velocity which can be sustained by an individual who can run for an extended period of time without fatiguing (Bergstrom, 2014).

The exercise which is performed is fuelled by a fixed quantity of work once the CV is exceeded, and the individual is considered to be in 'borrowed time' (Barker et al., 2006). The fixed amount of work is used by the anaerobic capacity and is deliberated related to an oxygen deficit-relating mechanism such as glycogenolysis, and an accumulation of fatiguing metabolites (Housh et al., 1991). This fixed work above the CV boundary is defined by the term  $D'$  or  $W'$  (running or cycling). At different intensities,  $D'$  may be depleted as a result of the amount of work being independent of the intensity at which exercise is performed (Housh, 2012). The more energy is needed to maintain the effort and therefore the external rate is expended quicker, when the power or velocity is further above the CT (Jones et al., 2007). The result from the difference between an individual's  $\dot{V}O_2$  max and CT is the

magnitude of external work (i.e  $D'$  or  $W'$ ). Sprint athletes tend to have a larger external work capacity ( $>25$  kJ) and a larger difference between CT and  $\dot{V}O_2$  max compared to endurance trained athletes which have a smaller capacity ( $<15$  kJ) and a narrower difference between CT and  $\dot{V}O_2$  max (Vanhatalo et al., 2010).

### 3.1.2 Application of CV

Any sporting situation where an important period is spent within the severe-intensity domain is related to the CV concept, thus an essential energetic contribution occurs from the constituents caused by  $W'$ . The CV concept has been adjusted for intermittent exercise and has also possible applications for interval training and team sports such as rugby and hockey. CV does not apply to sports that a single or only a few muscle contractions are involved (for example archery and field athletics), or where the limits set by the  $W'$  are unlikely to be significantly challenged in work-to-rest ratios (such as baseball, cricket) and also where the CP is not exceeded by the power output (e.g ultra-endurance events and golf) (Vanhatalo, Jones and Burnley, 2011). There is a lack of contemporary literature specifically addressing the possible application of CV to sport and so is worthy of investigation.

### 3.1.3 Fatigue and Critical Power (CP) mechanisms

Fatigue and exhaustion are described by the fundamental concept of CP. The ability to utilize  $O_2$  is the main physiological determinant of CP and this is dependent primarily on diffusion distance. Different tissue systems must increase their metabolic demand during exercise, and it is thought that each tissue system has their own CP. Cardiac and respiratory muscles for example, have higher relative CP compared to leg muscles because of the homeostatic functions that are provided by those tissues. Fatigue and exhaustion will occur during high intensity exercise if any of the contributing physiological system works above its CP (Walsh, 2000). A reduction in maximal voluntary effort is called fatigue and the voluntary inability to generate the required demand for the physical task is described as exhaustion (Bigland-Ritchie and Woods, 1984). According to Poole et al. (1988), CP represents a power output (PO) that can be maintained for 40-60 minutes before exhaustion occurs. Even if performance of the target intensity is not affected, fatigue can still accumulate or occur. Exhaustion will not occur if the PO is below CP and fatigue will not be persuaded. However, above CP, fatigue occurs and accumulates until the point which performance can no longer be sustained and then exhaustion takes place. The level of the PO above CP determines the amount of time before exhaustion occurs, therefore, the higher the PO, the shorter the TTE will be. A muscle can increase its absolute CP by expanding its size or by increasing its rate of oxygen delivery and utilization. A greater CP would result from larger muscle mass which would have a greater absolute critical force (CF). The other option would be to increase the oxygen flux and therefore the endurance capabilities of the muscle without altering the size of the muscle involved (Walsh, 2000).

### 3.1.4 Reproducibility

CV testing is considered as a more individualized method for training prescription in order to enhance performance or aspects of it and also health implications including risk of cardiovascular and metabolic disease reduction, all-cause mortality and quality of life augmentation. This power-time relationship or also called speed-tolerable duration relationship is based on a measurement of performance rather than a physiological index. As

a result, the performance attained through high intensity time to exhaustion effort is inclusive of fatigue as a complex occurrence, since multiple factors are involved with the relative importance of each. These factors are dependent on the fibre type composition of the contracting muscles, the intensity, type and duration of the contractile activity (Fitts, 1994). Compared to  $\dot{V}O_2$  max or blood lactate, they give a more confined marker which is not completely responsible for fatigue in sporting endeavours (Jones et al., 2010).

Based on a percentage of an individual's  $\dot{V}O_2$  max and HR max values, there are limitations considering the amount of time that they are able to exercise at the intensity threshold. For instance, some athletes may not be able to manage the prescribed intensity (e.g 95%  $\dot{V}O_2$  max) over a set period of time, whereas others may be capable of sustaining the intensity for longer to cause the same physiological stress on the body (Leclair et al., 2011). According to the total aerobic and anaerobic energy sources that are available, exercise prescription by CV may supply speed estimates or power outputs that are obtainable for the set time period.

A major disadvantage that CV testing may cause is that it needs repeated maximal efforts (3-4) where HR max and  $\dot{V}O_2$  max require only one. From a performance aspect, CV testing is designed for athletes and coaches to be able to receive all the benefits from repeated testing, which are also considered beneficial for a healthy motivated population (Housh et al., 1991). Testing involves 3-4 maximal efforts performed at different speeds based on the maximal speed that is achieved in the IET in a randomised order to span a time range of 3-20 minutes. In addition, the reproducibility of this concept remains unknown. It is therefore required to measure reproducibility of CV for optimal training and performance establishment which would be deemed beneficial for the development and acceptance of this concept.

### 3.1.5 Aim

The first aim of this study was to test whether CV and speed-tolerable duration are reproducible over two series of exhaustive tests on controls (non-runners) and runners. Specifically, the aim was to establish the CV and speed-tolerable duration in each subject by the use of four-time trials, and subsequently try to re-establish it with the same time trials. Therefore, assess whether this yielded a different CV and speed-tolerable duration relationship, in a set of controls (non-experienced to running) and runners (experienced to running). The hypothesis was that the reproducibility of the CV in male runners between the two series of testing are not significantly different.

## 3.2 Methods

### 3.2.1 Subjects

Eight (n=8) healthy active running male individuals and nine (n=9) healthy controls (non-runners) participated in this study. Activity diaries had to be completed by all participants. Runners were determined by how many times per week they performed running exercise. Recreational runners were involved in this part of the study who performed running exercise at least 5 times per week. Subjects were aged 18-35 years old, free from illness or other medical condition, non-smokers, free from using any protein supplements, anabolic steroids, drugs or other forms of doping. Subjects' characteristics are reported on table 1.

At least 10 visits to the lab were required over the period of 4 weeks per subject. Procedures involved a maximum of 3 tests a week with 48 hours rest between each trial at the same time of day ( $\pm 1$  hour). Instructions were given to refrain from strenuous training or exercise and alcohol 24 hours before testing, caffeine for 4 hours and food within 2 hours for all testing procedures, and this was consistent for all experimental tests.

Participants did not receive financial incentive or compensation for participating in this study.

### 3.2.2 Ethics

The MVLS College Ethical Committee for non-clinical research at the University of Glasgow ethically approved this study. Written informed consent was provided regarding the experimental procedures. Possible risks and benefits of participation were also explained to the participants and they also completed a pre-test health questionnaire and then they were cleared for participation. Subjects signed written consent before participating in the study and had the right for withdrawal at any stage without question.

### 3.2.3 Overview

Participation involved at least 10 visits in the laboratory where subjects were required to perform exhaustive treadmill tests.  $\dot{V}O_2$  max and  $\dot{V}max$  (the speed at which  $\dot{V}O_2$  max is achieved) were determined from the IET. For the remaining of the visits, at least 4 constant speed tests were performed at different percentages of  $\dot{V}max$  over 2 phases. No more than 3 experimental tests were performed in any given week.

### 3.2.4 Procedure

The gas analyser (Servopro 4100, Servomex, Texas) was calibrated before each test, using known concentrations and room air (Bergstrom, 2014). Before and after each trial, room and barometric pressure were recorded.

### 3.2.5 Equipment and Measurements

A motor driven programmable treadmill (PPS Med, Woodway, Weil am Rhein, Germany) was used for all exercise tests, at a 1% gradient. A gradient was used so that the lack of air resistance with indoor treadmill exercise was considered and match the energetic cost of the treadmill exercise with that of outdoor running (Jones and Jonathan H. Doust, 1996). A nose clip and a mouthpiece were worn at all tests. Mouthpiece was connected to a large 2-way



non-rebreathing valve (2700 series, Hans Rudolph, Shawnee, KS, USA), for respired gas collection (via a 1.5 m length of 3.5 cm diameter tubing) in a Douglas bag. For gas exchange variables calculation (especially  $\dot{V}O_2$ ), expired gas concentrations were collected with the analyzers (Paramagnetic (O<sub>2</sub>) and Infrared (CO<sub>2</sub>) analyzers; Servopro 4100 gas analyser, Servomex, Crowborough, UK) and gas volume (Dry gas meter; Harvard Apparatus, Edenbridge, UK). According to manufacturer's guidelines, the analyzers were always calibrated before each test, where precision analysed gases were used, which spanned the physiological range of inspired and expired gas concentrations. Post-test, the gas mixtures were re-sampled, to confirm the stability relation to the initial gas concentration. A short-range telemetry HR monitor (S610i, Polar Electro Oy, Kempele, Finland) was used in all experiments to determine HR which was recorded every 5 seconds, on the SRM Powercontrol III (SRM training system) data logger. After the HR was recorded on the data logger, it was possible to download the data later to a computer via an interface lead for further assessment.

### 3.2.6 Incremental Exercise Test (IET)

The IET started with a warm-up of 6 minutes at 8 km/hr where the speed increased 1 km/hr automatically every minute until exercise intolerance. In order to replicate the absence of air resistance, the treadmill endured at a 1% gradient to match the energetic cost of outdoor running (Jones and Jonathan H. Doust, 1996).

Expired air samples and HR were collected to be used as verification of maximal effort for the Constant Speed Tests. Maximal effort was achieved between 8-15 minutes. The verification criteria were HR max (220-age  $\pm$  10 bpm), respiratory exchange ratio (RER)  $\geq$  1.10, volitional exhaustion and  $\dot{V}O_2$  peak  $l/min^{-1}$  within 200 ml of maximal value (Gaesser and Pool, 1996).

Expired air samples were collected using Douglas bags continuously every minute until exhaustion was reached. The final two bags were analysed for maximal effort verification by the subject. When exhaustion was reached, the participant straddled the treadmill, despite strong verbal encouragement. Recovery was then performed, where the treadmill's speed was decreased down to 4 km/hr for approximately 5 minutes to ensure the participants' safety. There was  $\geq$ 24 hours difference recovery period between IET and the first trial of the Constant Load Tests.

### 3.2.7 Constant Load Tests

According to the maximal speed that was achieved in the IET, the Constant Load Tests were individualized to each subject, and were designed for exhaustion to be reached within 3 to 15 minutes (Moritani et al., 1981).

A 5.5 km/hr warm up was performed (brisk walk) for 6 minutes and then the treadmill accelerated to the pre-programmed speed for the trial. Two stopwatches were used to record the trial, in case of an error or malfunction while the participant ran until exhaustion. HR was measured continuously throughout the test. Expired air samples were collected in Douglas bags, when the participant was considered to be 2 minutes from exercise intolerance. Again, the participant straddled the treadmill when fatigued, and both stopwatches recorded the time which was then averaged.

Four Constant Load Tests were required to be completed by the participants at different speeds to measure their CV and D' whilst agreeing to Standard Error of Estimates (SEE) before they would progress into phase 2 with another set of 4 tests in a randomized order. This would provide two sets of CV and D' data in order to test the reproducibility. At least 8 Constant Speed Tests were completed with  $\geq 24$  hours difference from each trial.

### 3.2.8 Data and Statistical Analysis

A hyperbolic exponential decay relationship between time to exhaustion and speed resulted when each series of Constant Load Tests were plotted. Using the reciprocal of time (to exhaustion) and speed, a linear regression analysis was produced to identify the CV (Y intercept) and D' (slope of the line), estimated using least-squares linear regression of the linear Velocity/time<sup>-1</sup> relationship (i.e  $V = \left( \frac{D'(km)}{Time(s)} \right) + CV$ ) using Origin version 7.5 (OriginLab, Corporation, Northampton, MA, USA) (Vanhatalo et al., 2011).

Acceptable limits for CV and D' by SSE estimation, were  $\leq 2\%$  CV and  $\leq 10\%$  for D'. If the particular time trial test failed to meet those criteria, then the speed had to be repeated (Bergstrom et al., 2012).

For statistical analysis, paired samples t-tests were used to compare the test-retest between the controls against the runners. A Bland-Altman plot was used to determine the limits of agreement between Series 1 to Series 2 in CV and D' and Time. One samples t-tests of CV and D' data including absolute and absolute test-retest difference values was used for controls versus runners. Independent samples t-tests were used to compare the difference in controls with the difference in runners for CV and D'. A one-way ANOVA was conducted for  $\dot{V}O_2$  and HR data to compare with the IET and if any differences occurred then they would be identified by a Scheffe Post-Hoc test. Statistical significance was set at  $p \leq 0.05$  and the data were reported as mean  $\pm$  standard deviation (SD).

### 3.3 Results

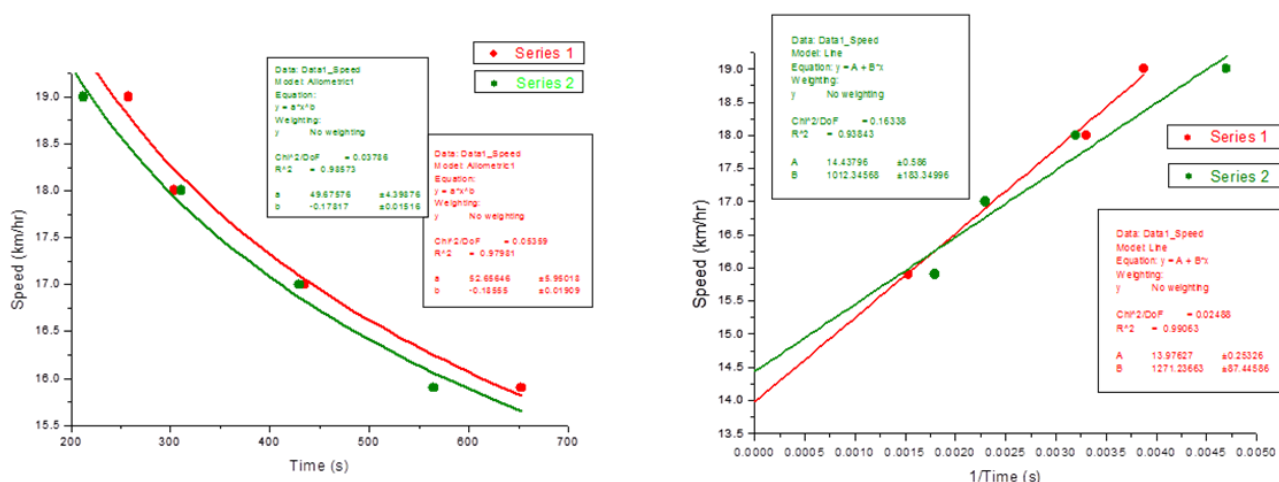
Table 1 shows the subject characteristics confirming that all the participants were young, healthy, recreationally active males. There was no significant difference ( $p > 0.05$ ) for any of the variables between runners and controls (age  $p = 0.794$ , height  $p = 0.415$ , weight  $p = 0.787$ ,  $\dot{V}O_2 \text{ max}$   $p = 0.206$ , HR max  $p = 0.732$ ).

Table 1: Subject Characteristics (n=17)		
	Runners (n=8)	Controls (n=9)
Age (years)	24.87 ± 4.76	24.33 ± 3.60
Height (cm)	180.55 ± 8.56	177.48 ± 6.45
Body mass (kg)	69.8 ± 6.38	70.59 ± 5.34
$\dot{V}O_2 \text{ max}$ (l/min)	4.20 ± 0.58	3.92 ± 0.58
$\dot{V}O_2 \text{ max}$ (ml/kg/min)	60.36 ± 7.46	55.60 ± 7.33
HR max (bpm)	196.87 ± 7.19	195.55 ± 8.24

*Table 1:* Subject characteristics shown as mean ± standard deviation. Mean values were given by the IET.

#### 3.3.1 CV&D'

Upon completion of the trials, the V-t and V-1/t relationships were plotted. An example of a subject X is shown in the figure below. The CV and D' were determined from the y-intercept and from the gradient of the line respectively from the V-1/t relationship.



*Figure 2:* Typical example of the Velocity-time (V-t) and V-1/t relationship from a test and a retest. Mean Critical Velocity (CV) and Distance Covered (D') of series 1 (Test) was  $13.97 \pm 0.25$  km/hr and  $1271.23 \pm 87.44$  m respectively. From the retest (Series 2), mean CV and D' were  $14.43 \pm 0.59$  km/hr and  $1012.34 \pm 183.35$  m.

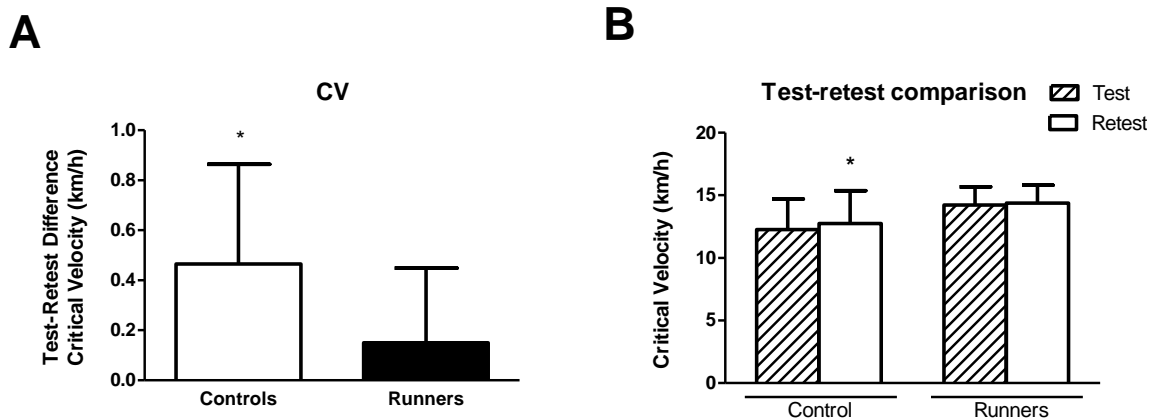
To properly assess reproducibility, we analysed both p-value and 95% CIs. From the paired-samples t-test it was found that CV was statistically different ( $p < 0.05$ ) for controls between the test and retest which means that CV for controls was not reproducible ( $p = 0.008$ , 95% CI = -0.771 and -0.157; see table 2). Mean CV for the test was  $12.27 \pm 2.42$  km/hr and for the retest it was  $12.73 \pm 2.63$  km/hr. When assessing for D', it was also found that between the test and retest for the controls, D' was statistically different ( $p = 0.013$ , 95% CI = 63.586 and 408.813) with mean D' for the test  $1124.46 \pm 342.135$  m and for the retest  $888.21 \pm 322.57$  m. The actual difference for the controls' CV was very small (0.46 km/hr) however it reached significance.

CV and D' for runners between the test and retest were not different ( $p > 0.05$ ) (CV  $p = 0.199$ , 95% CI = -0.400 and 0.100 and D'  $p = 0.146$ , 95% CI = -42.518 and 233.560; see table 2). Mean CV for runners for the test was  $14.21 \pm 1.46$  km/hr and for the retest it was  $14.36 \pm 1.43$  km/hr. Mean D' for the runners for the test was  $1035.08 \pm 376.93$  m and for the retest it was  $939.56 \pm 326.99$  m. Since there was no difference in both CV and D' between the test and retest for the runners it is suggested that these parameters are reproducible. The actual difference for the runners' D' was quite high (95.52 m), but significance was not reached.

Table 2: Mean absolute test-retest differences and analysis from one sample t-test					
	n	Absolute difference	95% Confidence Intervals		Significance p=
			(lower)	(upper)	
CV Controls	9	-3.61	-0.771	-0.157	0.008
D' Controls		21.01	63.586	408.813	0.013
CV Runners	8	-1.05	-0.400	0.100	0.199
D' Runners		9.22	-42.518	233.560	0.146

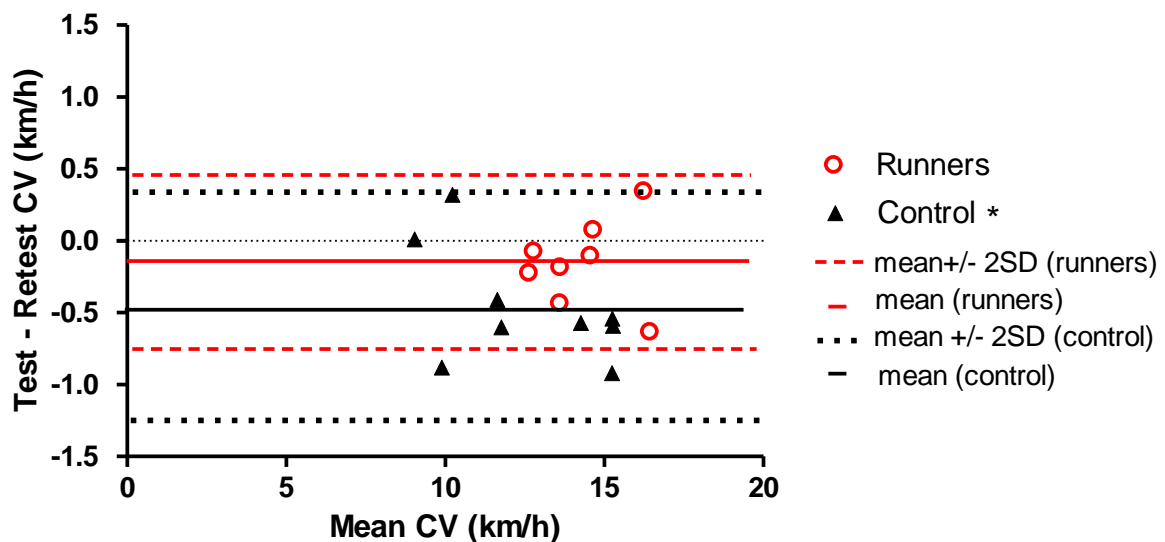
*Table 2: Mean absolute test-retest differences from one sample t-test for CV and D' from controls and runners.*

When analysing the independent samples t-test for  $\Delta CV$  and  $\Delta D'$  between controls and runners it was found that both  $\Delta CV$  ( $p = 0.089$ , 95% CI = -0.683 and 0.054) and  $\Delta D'$  ( $p = 0.166$ , 95% CI = -65.459 and 346.916) were not different between ( $p > 0.05$ ) the two groups.

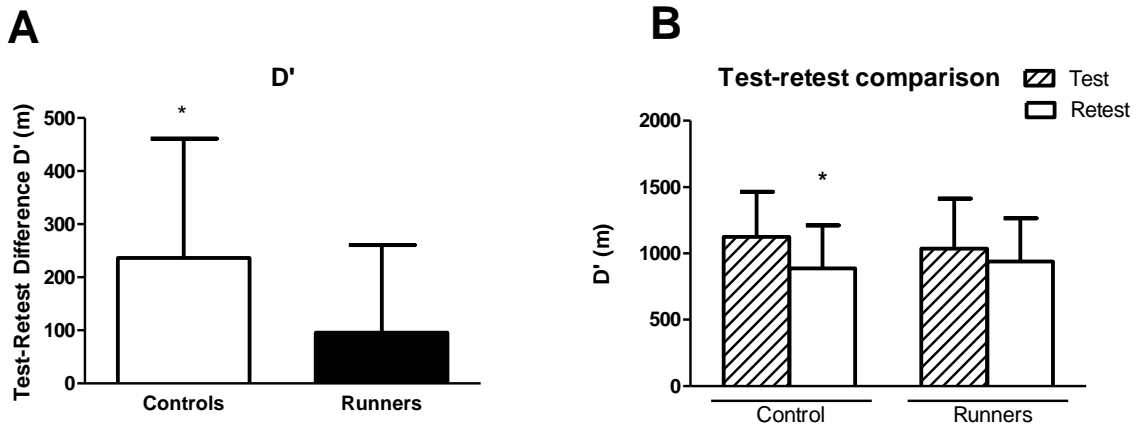


*Figure 3: In figure 3A it is shown that the difference of Critical Velocity ( $\Delta CV$ ) of the test-retest between the controls and runners was not significantly different. Figure 3B shows that the Critical Velocity (CV) was significantly different between the test and retest for the controls but not for the runners suggesting that CV is reproducible in runners.*

The spread of individual data around the mean can be observed by plotting test-retest values. Figure 4 displays how the values are spread out for controls and runners close to zero difference for CV with each individual each individual constant load test plotted. The higher CV for runners compared to controls can be observed with a split (to the right) in the data points.

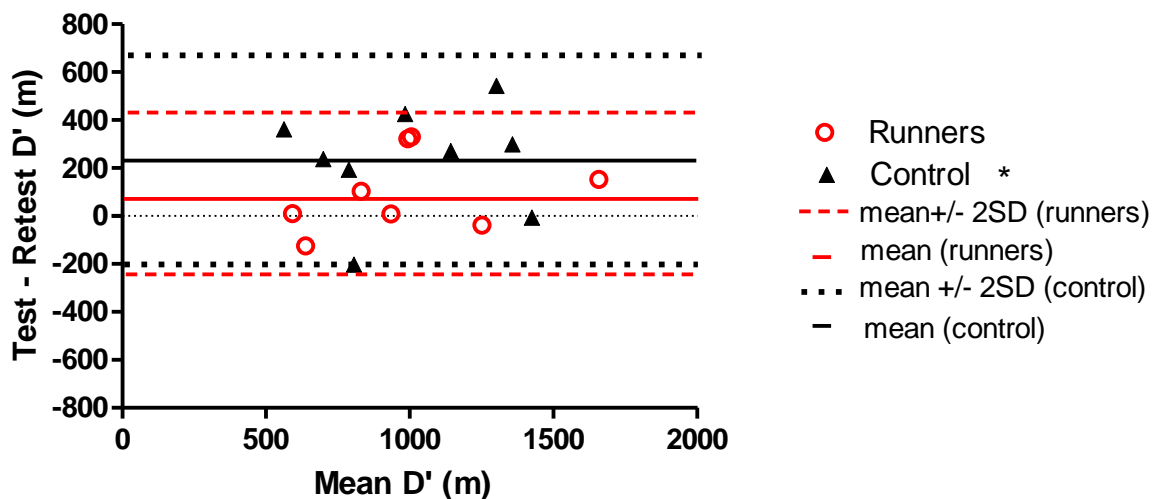


*Figure 4: Bland-Altman plot of difference between test and retest for Critical Velocity (CV) (km/hr). The limits of agreement are shown in the area between the positive and negative two standard deviations (2SD) and the mean difference is shown by the mean line. Since all the points are distributed near each other it is suggested that there was no significant difference for both controls and runners for CV.*

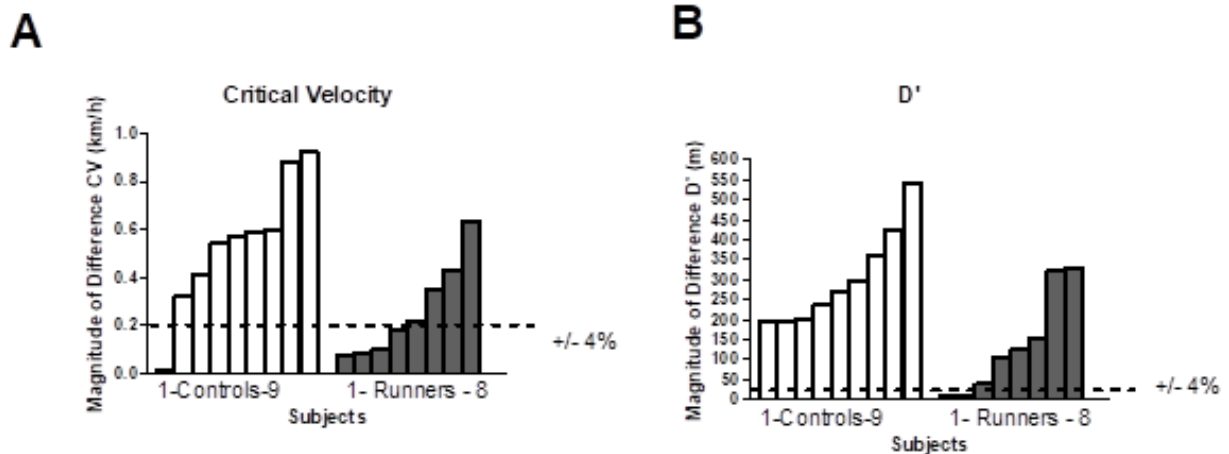


The spread of individual data around the mean can be observed by plotting test-retest values. Figure 5 displays how the values are spread out for controls and runners close to zero difference for  $D'$  with each individual each individual constant load test plotted. The mean test-retest for  $D'$  displays that both controls and runners have larger test compared to retest which is indicated by the positive mean lines. This is further confirmed on table 2.

*Figure 5:* In figure 5A it is shown that difference of Distance covered ( $\Delta D'$ ) was not significantly different between the test and retest for the controls and the runners. However, in figure 5B, Distance covered ( $D'$ ) was significantly different for the controls between the test and retest but not for the runners suggesting that  $D'$  is reproducible in runners.



*Figure 6:* Bland-Altman plot of difference between test and retest for Distance Covered ( $D'$ ) (m). The limits of agreement are shown in the area between the positive and negative two standard deviations (2SD) and the mean difference is shown by the mean line. Since all the points are distributed near each other it is suggested that there was no significant difference for both controls and runners for  $D'$  (m).

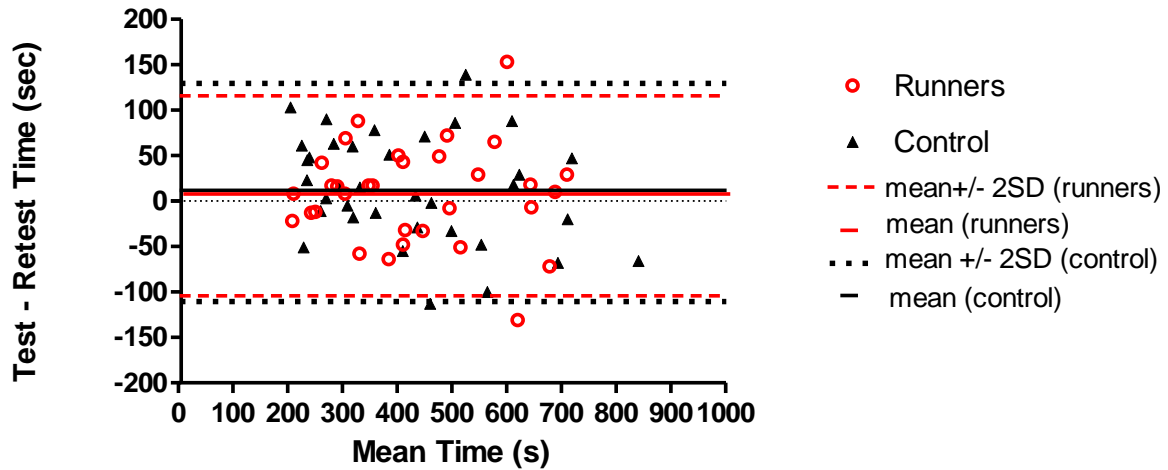


*Figure 7: The magnitude of difference (test-retest) of the CV (10A) and D' (10B) between the controls (1-9) and runners (1-8). Values that are below the arbitrary value of  $\pm 4\%$  are considered as reproducible. In this case, the runners show more reproducibility and consistency in their trials (test-retest) in both CV and D' compare to the controls.*

The mean absolute difference of test-retest values is displayed in table 2 below where CV and D' for controls are significantly different ( $p < 0.05$ ) when compared to the best theoretical response of reproducibility, zero. Controls were faster by 3.61 km/hr during retest however D' was higher by 21.01 m at test. For the Runners, CV was higher by 1.05 km/hr during retest however more distance was covered (9.22 m) during test.

### 3.3.2 Time

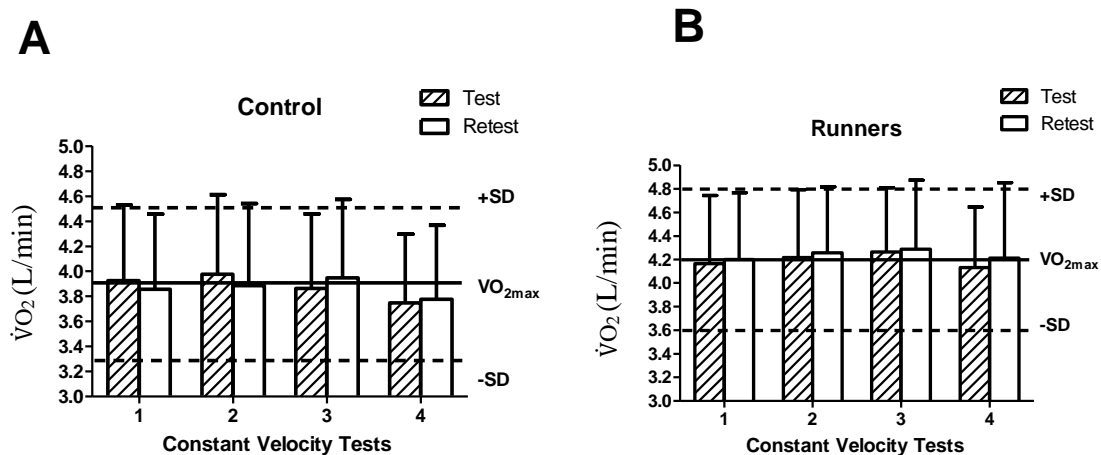
One sample t-test analysis for  $\Delta$ time confirms that there was no significant difference ( $p > 0.05$ ) between test and retest for both the controls ( $p = 0.160$ , 95% CI = -5.872 and 34.150) and the runners ( $p = 0.428$ , 95% CI = -11.965 and 27.527). Mean Time (t) for controls was  $14.13 \pm 59.14$  s and for runners was  $7.78 \pm 54.76$  s.



*Figure 8: Bland-Altman plot of difference between test and retest for Time (t) (s). The limits of agreement are shown in the area between the positive and negative two standard deviations (2SD) and the mean difference is shown by the mean line. Since all the points are distributed near each other it is suggested that there was no significant difference for both controls and runners for time.*

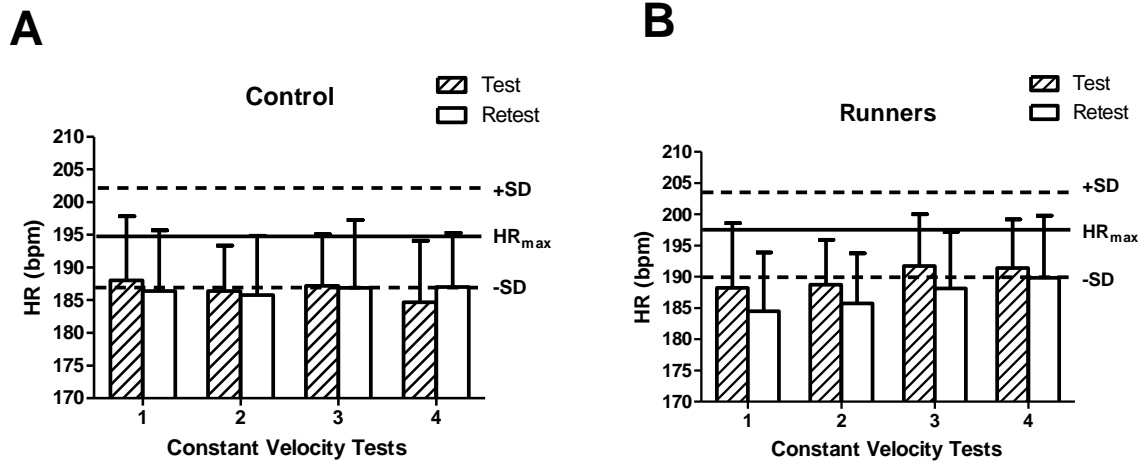
### 3.3.3 $\dot{V}O_2$ & HR from Time Trials

The  $\dot{V}O_2$  and HR that were produced from the time trials of the constant load tests, were then compared with the IET with the one-way ANOVA. It was found that there was no significant difference ( $p > 0.05$ ) for the  $\dot{V}O_2$  and HR of both the controls ( $p = 0.997$  and  $p = 0.368$ ) and the runners ( $p = 1.00$  and  $p = 0.208$ ), confirming that all the time trials were exhaustive.



*Figure 9: Shows the bar-plots of the Oxygen Uptake ( $\dot{V}O_2$ ) over the two series of Constant Load Tests. No significant difference was found between the test and retest for both the controls (9A) and runners (9B).*





*Figure 10: Shows the bar-plots of the Heart Rate (HR) over the two series of Constant Load Tests. No significant difference was found between the test and retest for both the controls (10A) and runners (10B).*

### 3.4 Discussion

The reproducibility of the CV and speed-tolerable duration relationship was assessed based on the protocol that was followed. Four Constant Speed Tests were performed in a randomized order, primarily based on the velocity that was achieved in the IET and subsequently on the time trials as they were undertaken, with the aim of achieving 4-time trials that scattered the duration of 2-3 minutes to ~15 minutes. The subjects ran until exercise intolerance in all tests. As set out by the purpose of this part of the study, testing the reproducibility of the CV concept was a requirement for the subsequent analysis. According to the obtained results, it is possible to say that the CV and D' were reproducible for the runners but not for the controls. Another finding was that when exercising at velocities above CV, the  $\dot{V}O_2$  max was always achieved despite the various speeds and durations used in the Constant Load Tests, suggesting that all the trials were exhaustive. This is considered as a major criterion for the appropriate CV assessment, which is in agreement with Hill and Ferguson (1999). In their study, Hill and Ferguson (1999) also showed that  $\dot{V}O_2$  max was always reached at velocities above CV and that at higher velocities, the  $\dot{V}O_2$  max was achieved in a shorted period of time.

The variability of the test-retest data for controls and runners for the CV and D' were shown in the Bland-Altman plots in figures 5 and 7. When analyzing this data, it was shown that there are no significant differences ( $p > 0.05$ ) in the CV and D' of the runners or the controls when compared to zero. Due to the best theoretical response of the test-retest, the comparison against zero was made, indicating no difference (thereby zero) between values which would indicate reproducibility (Atkinson and Nevill 1998) and no significant differences in either of the groups. Therefore, this shows that there were no systematic test-retest differences between the groups. However, it is more important to understand if a difference occurred or not and not at which direction this is in. In this case, it is not known which the correct value is (test or retest) because this part of the study did not test the validity of the CV.

#### 3.4.1 Participant differences

The  $\dot{V}O_2$  for runners would have been expected to be significantly larger than the controls, however that was not the case as shown on Table 2. Some of the controls that participated in this part of the study, were well trained individuals in different sports but not running, hence they were considered as non-runners but may have displayed a high  $\dot{V}O_2$  max. The running ability may be questioned between the runners and controls however, the subjects that were recruited in each group show differences in their running ability. Both the mean CV and D' for the runners were significantly different between the test and the retest, indicating that the runner group make better runners who are better able to control their own gait, than the controls which was expected (Moore, 2016). Some participants ran for over a minute longer in trials in either series, although the  $\dot{V}O_2$  value was  $\leq 200$  ml of their  $\dot{V}O_2$  max which was achieved in the IET. This might be associated with biological and mechanical variability, however other factors including training experience and psychological differences may have inhibited a consistent performance in controls where the runners may have benefited from their history and past practice of consistent running.

### 3.4.2 Open ended testing

There are two types of exercise test protocols to examine the influence of experimental interventions on endurance performance. These are time-to-exhaustion and time-trial exercise test protocols. Time-to-exhaustion is an exercise test which is performed at a constant speed or power output where the subjects are required to perform until exhaustion at a specific submaximal exercise intensities until that speed can no longer be maintained and this was the protocol that it was followed for this study. Conversely, an endurance performance test with a known endpoint is defined as a time-trial test. In this case, a set distance should be completed as fast as possible, or as much work as the subject can complete within a given time period. The distance or duration in a time-trial is known to the subject so that their work output could be adjusted and for them to be able to pace themselves towards this known endpoint. By having the athlete running to a certain endpoint over a distance as fast as possible with time trials it could improve the reproducibility of the CV, D'. Additionally, the ecological validity would be increased due to the fact that the time achieved would be less variable compared to testing to exhaustion. Laursen et al., (2007) found that there is lower variability in the time-trial compared to time-to-exhaustion tests which may be attributable to the fact that fatigue, boredom and lack of motivation can be more influential on performance during a time-to-exhaustion protocol. Exercise intensity can be increased or decreased during time-trial tests, in line with interactions between the athletes' perception of fatigue and external motivational cues. In contrast, during time-to-exhaustion tests, the exercise intensity is already established therefore the athlete chooses whether to continue or stop the trial completely.

Runners are more used to running to an endpoint, however this is dependent on a pacing strategy unlike the present method. The format of changing the Constant Load Tests to a time trial may be feasible area for enhancement in other CV studies and may better enhance the reproducibility in runners. These tests/trials are not always reproducible, hence why it is always required to check with the subjects every time they come in the laboratory that they are fully fit and prepared for an all-out bout. Their fatigue needs to be probed as well as their recovery, illness, training, especially from the day before testing. Scientists need to make sure that the subjects refrained from any exercise or alcohol on the day prior testing and also check their food intake to ensure that they are ready for this type of testing. If the coefficient of variation is too high, then the trial should also be repeated. For this reason, in the current study we employed a strategy whereby if the coefficient of variation was greater than 2%, the result was discarded, and the test repeated. However, in some occasions this was not the case, due to factors (especially time constraints or amount of repeated tests and potential subject's withdrawal from study) that influenced a participant's test and therefore, coefficient of variation was greater than 2%.

### 3.4.3 Experimental and biological variability

There is always going to be both experimental and biological variations affecting the consistency of these tests, which can only be controlled up to an extent. For practical application, the absolute differences of the test-retest for the CV and D' that can be considered acceptable are beneath 4% variation (Atkinson and Nevill, 1998). In our study, 1 out of 9 (11.1%) controls and 4 out of 8 runners (50%) magnitude of difference is under the variation line ( $\pm 4\%$ ) and can be considered as reproducible regarding their CV. Regarding the D', less reproducibility is shown with 0 out of 9 controls (0%) and 2 out of 8 runners (25%)

under the arbitrary 4% variability. Within the set limits, it could be considered that the subjects that are below the line of variability are reproducible. In this case, the runners were more reproducible than the control groups in both CV and D'. The variability (mainly biological) is unknown, as this 4% is subjective, and it is not deemed as an appropriate measurement of reproducibility, however, it may provide an understanding on the effects of the variability on the absolute test-retest values. Further research is required to determine a consistent and an acceptable measurement error before the variables are deemed as reproducible. According to Atkinson and Nevill (1998), if the measured values are not within an acceptable measurement error, then the validity of the concept which is examined will never occur. Therefore, the validity of the CV concept needs to be further examined.

#### 3.4.4 Application of CV concept

The CV concept can be used by coaches, to improve their athlete's training and performance, as an exercise intensity prescription. Accurate determination of exercise intensity at strenuous exercise is important in prescribing training programs for elite athletes and individuals. In exercise that is performed in the 'severe' domain including interval training programmes (HIIT), exercise intensity prescription is essential as this type of exercise involves completion for a short duration at intensities above CV. D' could also be applied, to predict the distance that runners can cover before they reach exhaustion. Sports scientists and coaches may prescribe this type of exercise in order to improve the aerobic capacity of the runners.

According to Ferguson et al. (2013), the CV and speed-tolerable duration can be used to optimize High Intensity Interval Training (HIIT). The main problem with using CV to predict HIIT would be the recovery, specifically how much of D' can be expected to be replenished in the active recovery periods. They demonstrated that the Work Rate (WR) that was derived from the speed-tolerable duration relationship which leads to the limit of tolerance in 6 minutes, was optimal. This provided the required balance between D' depletion and repletion during recovery. They suggested that the exercise intolerance is dependent entirely on the extent of D' recovery, because the CV remains the same after fatiguing exercise. This remains unknown, but a suggestion has been that 50% of D' may recover in 4 minutes (Ferguson et al., 2010). In the study of Ferguson et al. (2013), they suggested that as D' recovery averaged at 50%, leads to the confirmation of the assumption that the extent of D' recovery is not varying between repeated bouts. Therefore, the WR was derived from the speed-tolerable duration relationship which leads to the limit of tolerance in 6 minutes gives the appropriate WR for normalization of the intensity of 4x4 min HIIT to the heavy-intensity domain. This study also gives an approach where exercise intensity can be normalized, and supra-CV work can be maximised for every participant during 4x4 min HIIT. HIIT strategies may therefore have important implications to maximise improvements in physiological functioning.

Another use of the CV concept would be as a pacing strategy in competitive performance. Pacing strategy is defined by Abbiss and Laursen (2008) as "the distribution of an energy source over a period of time". At any point during an endurance event, if a runner's velocity is decreased below that of its CV, then its performance is decremented. CV gives an index of aerobic capacity and the ability of an athlete to resist fatigue can control this concept. The speed-tolerable duration relationship of the athlete can be used for regulation when D' would be depleted and therefore when the athlete would reach fatigue. In order for the athlete to complete the distance in the most efficient way, the pace needs to be modified so that it is evenly spread across the race. Voluntary fatigue is not considered by the CV concept,

meaning that the athlete chooses when to stop, when physically could run for longer. This does not greatly affect the elite athletes due to the fact that they know how to use their mental toughness to push themselves to the limits. Recreational or amateur athletes might not know their limit therefore their volitional fatigue would occur prior depletion of their energy resources.

#### 3.4.5 Practical Application of CV

According to Vanhatalo et al., (2011) “the CV concept can be applied to any dynamic activity including running, rowing, swimming and continuous and intermittent isometric exercise where the analogues of power (speed/velocity, force, torque) are substituted as appropriate”. Activities including swimming, strength and conditioning training and bench press have also been studied.

In a study that examined the relationship between anaerobic CV and short distances performances in the four swimming techniques in young swimmers it was found that the CV assessed by short testing distances was similar to the 200 m swimming event velocity in four conventional swimming techniques. It is further suggested that CV can be used as a race-pace training reference since linear relationships were found with CV and performance in the 50 m and 100 m (Marinho et al., 2011).

Another study examined the effects of a sports specific maximal 6-week strength and conditioning program on CV, anaerobic running distance and 5-km TT performance. It was concluded that 6-week resistance training program (heavy load 80% 1-RM) in combination with endurance training significantly improved 5-km TT performance by 3.62%. Anaerobic running distance was decreased with non-significant changes in CV, however CV increased 2.98% after a combination of strength and endurance training. Coaches and athletes should therefore be encouraged to combine heavy resistance strength and conditioning training for a minimum of 6 weeks when preparing for races (Karsten et al., 2016).

The 3-parameter CP model derived from cycling and running, to performance and bench press exercise was also applied in another study. The model was applied in 1RM and multiple repetitions to failure at different submaximal weights. It was concluded that by using the 3-parameter CP model the numbers of bench press repetitions to failure at submaximal level can be successfully predicted. This model can be used to estimate the 1RM bench press value of the lifter also to estimate and the aerobic, anaerobic and maximal parameters that describe these abilities of the lifter (Morton et al., 2014).

CV concept is therefore considered as a valuable assessment tool for performance monitoring in sport due to the fact that is deemed as a useful framework so that the physiological mechanisms underlying exercise tolerance can be studied. Future challenges of this concept would be to fully understand the multiple and relevant determinants of both CV and D', clarifying methods for their assessment and in establishing interventions which would positively influence these parameters and therefore enhance performance.

#### 3.4.6 Limitations

Few limitations could be mentioned that occurred in this part of the study. This was a laboratory based rather than a field-based study, where the laboratory has very controlled environmental conditions. These conditions may not be replicating the outdoor running in a

very precise way. The treadmill was set to a 1% gradient to match the energetic cost of treadmill exercise with that of the outdoor running however other environmental factors such as hill gradients and weather conditions were not assessed. Therefore, it is not known whether the environmental factors could have an impact on the V-t relationship.

Another limitation was that the training regimes and dietary intake of the subjects were not controlled. According to the information that the subjects were given, they should not exercise the day before the test and keep rested between subsequent tests. This was because, if exercise above CV was performed prior to testing, could have negatively influenced the replenishment of D'. And if D' is not fully restored after exercise, the V-t relationship could have been decremented.

#### 3.4.7 Conclusions

It can be concluded that by using the IET followed by four Constant Speed Tests over two series of exhaustive tests at different speeds in a randomized order, it is possible to measure the V-t relationship, CV and D'. It was shown that the runners are more reproducible in replicating their CV and D', since there was no significant difference on their CV and D', compared to the controls where a difference was found. In addition, at velocities above CV,  $\dot{V}O_2$  max was always attained showing that the exercise intensity was appropriate to reach  $\dot{V}O_2$  max at each Constant Load Test. In accordance to this finding and the CV definition, exercise leads to fatigue and exhaustion. A runner of a higher standard who may be more familiar to exhaustive efforts and may be mentally tougher, with greater motivation may be better able to reproduce these efforts compared to a club/recreational level runner. However, the recruitment of these athletes is practically more difficult. Another series of testing would be ideal which would involve a higher standard athlete, in order to accept the CV concept as reproducible. Then the CV concept could be used for exercise intensity prescription where an acceptable variability limit would also be required.

## **4.0 New Zealand Blackcurrant Extract on Health and Performance**

### **4.1 Introduction**

#### **4.1.1 Overview**

Anthocyanins are becoming very popular as supplements within sports nutrition. Anthocyanins are pigments within berries and also other fruits and vegetables. Anti-oxidative and anti-inflammatory actions of anthocyanins may improve recovery from exercise. Effects on blood flow, metabolic pathways and peripheral muscle fatigue or combination of all these, are the mechanisms with which anthocyanin intake may result in performance enhancement. High intake of polyphenols is associated with lower risk for multiple diseases according to epidemiological studies. There are 4 groups of polyphenols based on chemical structures including phenolics, flavonoids, stilbenes and lignans. Within these groups, there are also classes. A class of flavonoids involves the anthocyanins (Cook and Willems, 2018).

The dietary intake of the main anthocyanins includes glycosides of their respective aglycones, which are the pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin. Anthocyanins are water soluble and act as natural pigments, causing the purple, blue, red and orange colours of leaves, flowers, fruits and vegetables. Blackcurrant (BC) contains high levels of anthocyanins including cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-glucoside and delphinidin 3-rutinoside. The purple/black colour of the fruit is given by the high content of anthocyanins. BC is described as a spineless, stout, woody, deciduous shrub with toothed-edged, abundant leaves. BC is rich in Vitamin C (including other vitamins and minerals), 7 polysaccharides, flavonoids, and at least 15 different phenolic acids (proanthocyanidins and anthocyanins) (Ehala, Vaher and Kaljurand, 2005).

For centuries, traditional healers have been using BC for different conditions including arthritis, kidney stones, liver disease, fatigue, stomach and bowel disorders and inflammations (Leventhal, Boyce and Zurier, 1994). Chinese medicine used BC or also called *Ribes Nigrum* as a diuretic, diaphoretic and febrifuge and also as an ingredient in juices, wines, jams and nutraceuticals. Scientists have started to investigate this fruit because of its healing and protective benefits. BC does not develop in hot or dry climates and it is native to the Midwestern United States. New Zealand is considered to be the most ideal place for the substance's growth due to the moderate climate throughout the year.

#### **4.1.2 Mechanisms on Health**

BC contains high levels of antioxidants against various conditions including diabetes (Prior et al., 2008), obesity, cardiovascular disease (Wallace, 2011), cancer and the degenerative diseases of ageing. The antioxidant activity may delay or counter some of the mechanisms or processes in the body that can lead to those diseases. The endothelial function is altered by an up-regulation of the enzyme called endothelial nitric oxide synthase (eNOS) and this is where the effects of anthocyanins may be attributed (Speciale et al., 2014). This is suggested to be completed when the vascular smooth muscle cells relax, and the blood vessels vasodilate (Suhr et al., 2013). These are two mechanisms that may be independent of each other, and may sometimes also be closely linked, 1) oxidative stress and antioxidant defence and 2) endothelial generation and release of nitric oxide (NO). BC seems to maybe affect both. Both mechanisms would also be advantageous and may combat the same diseases, quite possibly

better than one mechanism alone. BC has demonstrated many health benefits including increased urine pH (alkalinizing effect), oxalic and citric acid secretion (Kessler, Jansen and Hesse, 2002). It also inhibits gram-negative bacteria (Puupponen-Pimia et al., 2001), influenza virus type A and B in canine kidney cells and suppresses the growth of a virus (Knox et al., 2003). Plaque formation, replication in cells and the herpes simplex virus type 1 attachment on cell membranes were all inhibited in another in vitro study (Suzutani et al., 2003).

#### 4.1.3 Effects on Inflammation and Immune Response

There was a direct effect of BC on inflammatory response when measuring the biomarker of inflammatory response in the blood Interleukin-17 (IL-17). Healthy overweight subjects consumed a high fat meal and showed production of IL-17 an hour later and remained high for up to 8 hours. An equivalent high fat meal with a fruit juice drink which contained BC and other fruit, inhibited the IL-17 production (Peluso et al., 2012). In other words, albeit other components of the fruit juice may also have contributed, BC appears to have resulted in the opposite inflammatory response to a high fat meal, reduced inflammation rather than the expected increase in signs of inflammation.

Different aspects of performance showed positive effects with BC supplementation. Immune response was enhanced according to a study which was conducted in 2009, which also resulted in an increased ability of regular exercise including increased lung efficiency and capacity, cardiac output and arterio-venous oxygen difference. In a study where capsules were taken pre and post exercise for 3 weeks (1/3 cup berries and 240 mg anthocyanin) it was found that the ability of plasma to suppress inflammatory responses was increased and the levels of biomarkers of oxidative stress in plasma were significantly lower. These benefits involve faster tissue repair, recovery and performance. By combining immune protection and muscle damage and soreness reduction, the natural benefits of exercise can be augmented (Lyall et al., 2009).

In a double-blind crossover trial, 12 subjects were given a single dose of BC anthocyanin extract and showed a dose-dependent effect on lowering the dark adaptation threshold, in other words how the eye recovers its sensitivity in the dark, after exposure to bright lights (Nakaishi et al., 2000). The regeneration of rhodopsin was also enhanced by BC anthocyanins and resulted in sustained and progressive relaxation of bovine myopic ciliary muscle, which then improved vision (Matsumoto et al., 2003).

Inhibition of proliferated breast cancer cells and colon cancer cells was also observed in another study (Olsson et al., 2004). The results vary in the literature considering the effects on lipid profile showing both positive and negative effects on cholesterol and triglycerides (Nielsen, Ramsussen and Mortensen, 2005).

#### 4.1.4 Pain and Muscle damage

There was a positive effect on chronic musculo-skeletal pain (fibromyalgia) according to another study where anthocyanins from berry and grape extracts were used. Subjects used the anthocyanin powders for a period of 3 months. Fatigue was reduced as well as sleep disturbance, and general health was increased suggesting that anthocyanins may alleviate the suffering from chronic conditions (Edwards et al., 2000).



Any pain caused by exercise may also be prevented by BC juice consumption. When subjects took a pill pre and post exercise, they suffered less inflammation and muscle damage according to a study. It was suggested that the compounds of flavonoids that make part of the berries can protect the body from the stress that exercise causes. The subjects who took the pill pre and post daily moderate exercise reported less signs of inflammation, muscle damage and oxidative stress. A combination of fruit extracts and exercise may be therefore be beneficial to human health (Derbyshire, 2010).

#### 4.1.5 Physiology and Performance Benefits

##### 4.1.5.1 Muscle damage/ Stiffness, Blood Flow, Inflammation

Muscle stiffness was decreased after BC supplementation, resulting from a reduction in muscle fatigue and an increase in peripheral blood flow. In this study, the subjects consumed 50 g anthocyanin and a typing work was performed for 30 minutes. The total and oxygenated haemoglobin were significantly higher in the BC group whereas in the PLA group, a significant stiffening of the trapezius muscle was reported. However, it was shown that the final stiffness did not differ between the groups (Matsumoto et al., 2004).

Improved blood flow was also demonstrated based on 3 clinical trials involving cycling performance, where lactate accumulation was lower and thus resulted in improved recovery, cardiovascular function and performance (Willems et al., 2014). After 8-day supplementation (twice a day), BC reduced inflammation and muscle damage and antioxidant capacity. The antioxidant capacity was reduced in the PLA group but was maintained in the BC group. However, BC consumption did not reduce the leg muscle soreness but returned to baseline at a faster rate of a whole day compared to the placebo group. It was suggested that BC may be used as a natural food alternative to anti-inflammatory and analgesic drugs following high-intensity eccentric exercise (Hutchison et al., 2014).

##### 4.1.5.2 Performance ( $\dot{V}O_2$ max and Lactate)

Different types of BC extract were examined to test whether they can improve various types of exercise. New Zealand Blackcurrant (NZBC) increased the total running distance by 10.6% (improvement of 411 m) and distance covered during sprints by 10.8%, (improvement of 277 m), and lactate clearance was greater in 64% of the participants by 30 minutes. Participants were thirteen physically active males who consumed one capsule per day which contained 300 mg CurraNZ BC (105 mg anthocyanin) or PLA. Lactate was higher at exhaustion and the lactate clearance was improved after 15 and 30 minutes of passing recovery with BC supplementation. Physiological responses such as HR,  $\dot{V}O_2$  max, La, and RPE were shown to be normal during high-intensity intermittent exercise. It was concluded that due to lactate tolerance and improved recovery, sports that involve high-intensity intermittent exercise capacity may improve performance with the BC extract (Perkins et al., 2014).

NZBC also improved a 16.1 km time trial (TT) performance by 3.6% in 78% of the participants with higher lactate values at the end of the TT. Participants consumed 1 capsule per day 300 mg BC or placebo for 7 days, with washout period of 2 weeks. In 89% of the participants the lactate clearance was greater in 20 minutes. Metabolic and physiological responses during low and moderate intensity cycling were also normal due to the fact that

there were no changes in HR, La, cycling economy and fat and carbohydrate oxidation (Willems et al., 2014).

Six grams per day of Sujon BC powder (a different type of BC supplementation in the form of powder) resulted in augmented stroke volume by 25% and cardiac output by 26% and in decreased total peripheral resistance by 16% in 78% of the participants. Participants consumed Sujon BC powder or PLA for 7 days. Blood pressure and heart rate remained unaffected. It was suggested that nutrients and clearance of metabolites for the resting skeletal muscle may be affected by these observations and also strategies regarding the recovery for sports performances might be supported by the effects produced by the Sujon BC on cardiovascular function (Willems et al., 2015).

A study by Willems et al. (2014) showed that 77% of the triathletes decreased their maximal oxygen uptake by 14% with Sujon BC supplementation. The exercise intensity might be affected at lactate indicators (onset of blood lactate accumulation at 4 mMol/L [OBLA] and maximal oxygen uptake) due to increased peripheral blood flow which is caused by BC. Six grams per day Sujon BC powder or PLA were consumed by 8 males and 5 females. BC intake caused complete shift of the lactate curve and the power at OBLA was greater by 6% with 85% of the triathletes showing an increase but no effect on HR and  $\dot{V}O_2$  max. Production and removal mechanisms cause the blood lactate to accumulate exponentially, especially during incremental intensity exercise. The increase in peripheral blood flow by anthocyanin may benefit the lactate removal mechanisms and may also be enhanced by heart, liver, kidney and skeletal muscles. According to Emhoff et al., (2013), glycogen sparing can be the result of improvement of intracellular lactate oxidation and conversion into glucose. Endurance athletes might be benefited from Sujon BC powder to enhance their performance due to the delay in OBLA that can be caused during prolonged high intensity exercise at both competition and training.

#### 4.1.5.3 Recovery

It was suggested that BC intake may aid post-exercise recovery from high-intensity exercise which is influenced by peripheral circulation and venous return. The effects of BC extract on blood flow might also enhance high intensity exercise for example repeated sprints which are common in particular team sports. Fatigue is related with phosphocreatine (PCr) degradation and metabolite accumulation during high intensity intermittent exercise, therefore, interventions that would minimize these effects would be beneficial for this type of exercise (McMahon and Jenkins, 2002). Increased muscle oxygen availability may enhance the PCr resynthesis during the recovery of intermittent exercise. Lactate clearance may be benefited by blood flow and its distribution, mainly via gluconeogenesis and oxidation (Brooks, 2007). BC extract may then contribute in PCr stores maintenance and metabolite accumulation reduction, where the onset of fatigue is delayed. Therefore, this will lead to post-exercise recovery improvement and performance of repeated sprints enhancement (Perkins et al., 2015).

#### 4.1.5.4 Substrate Oxidation

The effects of NZBC on substrate oxidation, cycling time trial performance and plasma lactate responses after the time trial were examined by a study by Cook et al., (2015) in trained cyclists. They found a significant increase of 2.4% in time trial performance and this

means an important advantage to endurance athletes because this increase did not occur with alterations of training or diet before the trial but because of the higher power output across the time trial. The improvement may not be attributed to a chronic training effect after 7 days of supplementation with BC and training. The short duration intake altered the training adaptations rather than the physiological responses resulting on the enhancement of high-intensity performance which involved a large aerobic component. Improved endothelial function is caused by BC supplementation due to the large amounts of delphinidin and other anthocyanins in the BC extract. Increased endothelial cells and reduced breakdown by NO free radicals may lead to promotion of NO (Nagai et al., 2002).

In the study of Cook et al. (2015), fat oxidation was also increased by 27% at BC intake at moderate and low intensity and CHO oxidation was lowered. The reason behind the elevated fat oxidation could be that many pathways acting together in combination. This suggests that the availability of NO, blood flow measures and lactate kinetics should be assessed with BC intake prior, during and after exercise especially exhaustive exercise where the lactate levels are augmented.

#### 4.1.6 Mechanisms on Performance

Repeated high-intensity exercise causes peripheral muscle fatigue which often leads to accumulation of metabolites and by-products of metabolic pathways, decreased energy supply as well as changes in ionic concentrations (Girard et al., 2011). This type of exercise lowers the intracellular muscle pH (acidosis) in addition with muscle excitability along the t-tubular membranes and increases extracellular potassium and intracellular sodium and chloride concentrations (McKenna et al., 2006). Consumption of BC can postpone peripheral muscle fatigue by increasing the levels of intracellular acidosis, therefore it can counteract the oxidative stress and the production of reactive oxygen species (ROS) because of its antioxidant activity (Morales-Alamo and Calbet, 2013).

Exercise-induced oxidative stress has been implicated in muscle damage, immune dysfunction and fatigue, due to the accumulation of ROS (Powers and Sens, 2000). Vitamin C or other antioxidant supplementation can protect athletes from the negative effects of exercise which are caused by oxidative stress (Schwenk and Costley, 2002). Regulation of different intermediate kinases and phosphatases are the outcomes of the ability of reactive oxygen species, when the expression of proteins that includes training adaptation is involved. Increased kinase activity and decreased phosphatase activity result from ROS activity, which leads to activation of transcription factors. When the transcription factors are activated then the mitochondrial growth factors are increased as well as the cell survival proteins, whereas the muscle atrophy along with the proteins involved in cell death signalling pathways are decreased. This is the reason why ROS are considered as a significant trigger for training adaptations to occur (Braakhuis et al., 2013). The expression of proteins' RNA related to exercise adaptation including mitochondrial biogenesis and antioxidant enzymes, is blocked by antioxidant supplementation and this may result in negative consequences by weakening the adaptations to training.

Changes in vascular function, including improvement of the endothelial function and proliferation in blood vessels, are mechanisms with which polyphenols may result in performance benefit. It is suggested that performance enhancement by polyphenols is likely

to occur when greater training load takes place, which is the case in the faster runners (Braakhuis et al., 2013).

BCs have high antioxidant activity and are rich in flavonoids, which offer protection against exercise-induced oxidative stress (Skarpanska-Stenjbörn and Pilaczynska-Szczensiak, 2006). Gomez-Cabrera et al. (2008), suggested that the mechanisms for the decrements in performance may be a result of sub-optimal mitochondrial biogenesis and/or the large dose of antioxidants acting as a pro-oxidant and hampering muscle force generation or excessive recovery. This statement suggests that athletic performance can be decremented by a high dose of antioxidant nutrients (Ristow et al., 2009).

It is therefore important to take into consideration the correct way to supplement athletes with those ergogenic aids combined with the right amount of training load. Consequently, the large doses should be avoided on types of exercise including high intensity and more specifically CV testing which requires repeated multiple high intensity testing to exhaustion.

#### 4.1.7 Aim

The aim of the study was to examine the effects of NZBC on CV and speed tolerable-duration relationship during running and resting metabolism on recreationally active males over multiple exhaustive tests. By using four-time trials, the aim was to establish the CV and speed tolerable duration relationship and then try to re-establish it with the same time trials, in order to test whether a different value of CV was produced between intervention (BC supplementation) and non-intervention (PLA intervention).

## 4.2 Methods

### 4.2.1 Participants

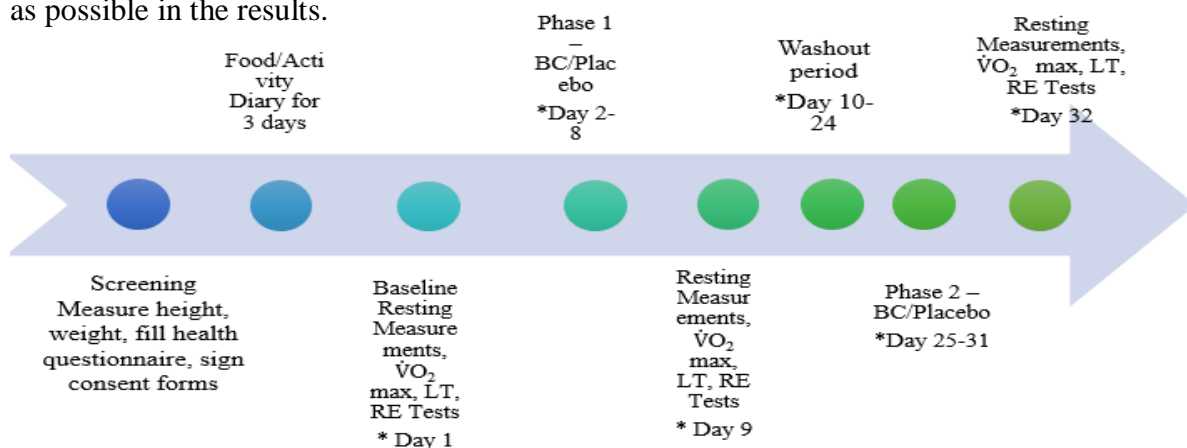
On this randomized, double blinded, cross over study, 7 (n=7) subjects (different individuals to the previous study) volunteered to take part (recreationally active males, aged 18-45 years old) who were free from illness or any other medical condition. Participants should be non-smokers and be refrained from any nutritional supplements (protein, steroids, drugs or any other forms of doping). Non-runners were recruited for the study in response to lack of availability of a running cohort. Given the results from the first part of this work, runners were the preferred cohort however difficulty in recruiting runners at the time of this part of the study meant non-runners were approached. Whilst the participants weren't part of a running club, they were familiar with treadmill running as part of their training routine. They first attended a screening session where all the experimental procedures, risks and benefits were explained to them. Height and weight were recorded by using a wall stadiometer and a calibrated balance. Then, they completed a health-questionnaire and signed an informed consent. Participants were recruited from the University of Glasgow (Athletics Club, Sport and Recreation) and also from Crossfit Glasgow.

### 4.2.2 Ethics

All procedures were approved by the University of Glasgow College of MVLS Research Ethics Committee for non-clinical research. All data, results and information would be kept confidential. Volunteers did not receive any payment for participation and had the right of withdrawal at any stage of the study without question.

### 4.2.3 Study Design

Volunteers were asked to attend at least 11 occasions at approximately the same time of day (+/- 2 hours). There was at least 24 hours difference between sessions and each subject attended testing in no more than 3 times in any given week. No strenuous exercise should be performed as well as no alcohol intake, no caffeine and no food intake should be consumed 24 hours prior testing. The study was comprised of 2 phases (BC or PLA) separated by a 14 day of wash-out period. All subjects were fit and trained enough to be able to sustain the exercise protocols. Three days prior to every metabolic day subjects were asked to keep and replicate a food and activity diary in order to standardize their intake and avoid any variations as possible in the results.



*Figure 11: Study's projected timeline. Twelve participants have been screened but only seven of them were involved in the study. There was no supplementation on baseline measurements involving resting measurements and incremental exercise test (IET). Resting measurements included ventilation hood (REE), venous blood samples collection and anthropometric measurements (Bodpod).*

#### 4.2.4 Equipment and Measurements

A motor driven programmable treadmill (PPS Med, Woodway, Weil am Rhein, Germany) was used for all exercise tests at a 1% gradient so that the lack of air resistance with indoor treadmill exercise would be considered and to match the energetic cost of the treadmill exercise with that of outdoor running (Jones and Jonathan H. Doust, 1996). A nose clip was worn as well as a mouthpiece which was connected to a large 2-way non-breathing valve (2700 series, Hans Rudolph, Shawnee, KS, USA), to collect respired gas (via a 1.5 m length of 3.5 m diameter tubing) in a Douglas bag. Gas exchange variables were calculated by measuring expired gas concentrations (Paramagnetic (O<sub>2</sub>) and Infrared (CO<sub>2</sub>) analyzers; Servopro 4100 gas analyser, Servomex, Crowborough, UK) and gas volume (Dry gas meter; Harvard Apparatus, Edenbridge UK). According to manufacturer's guidelines, gas analyzers were calibrated before each test, where precision analysed gases were used to span the physiological range of inspired and expired gas concentrations. Room temperature and barometric pressure were also recorded prior to each test. A short-range telemetry HR monitor (S610i, Polar Electro Oy, Kempele, Finland) was used to determine the heart rate (HR) in every test. The SRM Powercontrol III (SRM training system) data logger was recording the HR, and then the results could be downloaded to a computer via an interface lead for analysis.

#### 4.2.5 Protocols

##### 4.2.5.1 Supplementation

Participants were instructed to take one capsule every morning with their breakfast, apart from the first baseline measurements where there was no supplementation. Supplementation started on the day before their first trial and continued every day until the first phase was finished. The first phase was followed by a 14-day wash-out period. Then, participants repeated the procedure for the second phase. On the day of the tests, subjects were overnight fasted and took one capsule 30 minutes prior on their arrival at the lab. Capsules contained 105 mg anthocyanin per dose of 300 mg CurraNZ, administered as one capsule per day (CurraNZ, Health Currancy Ltd, Surrey, UK) or PLA (300 mg microcrystalline cellulose M102) administered also as one capsule per day. The administered dose was according to manufacturer's guidelines as there is not yet a consensus from the existing research for an optimal dosing strategy for NZBC. This research has been described in 4.1.5.

##### 4.2.5.2 Resting Energy Expenditure (REE)

Resting Energy Expenditure (REE) was measured with the Quark CPET System, in canopy mode, which was calibrated before any test. The participant was asked to lie down on the bed in flat position for 10 minutes to relax before the test commenced. A ventilation hood was then placed over their head. The hood was connected to the C-PET by using the corrugated tubing and a fresh adaptor. The test was conducted for 25 minutes, where the last 15 minutes were averaged to be used for the results. To ensure that the participant was in a rested state,

the Respiratory Exchange Ratio (RER) or 'R' value was monitored consistently throughout the test and should be below 1. RER is the ratio between the amount of carbon dioxide (CO<sub>2</sub>) that is produced in metabolism and the amount of oxygen (O<sub>2</sub>) that is used. Exhaled gases to room air comparison determines this ratio. The R value is used for the respiratory quotient (RQ) estimation, which is an indicator of which fuel (carbohydrate or fat) is being metabolized so that the body is being supplied with energy. Metabolism needs to be in a steady state in order for this estimation to be valid.

#### *4.2.5.3 Bodpod*

Indices of body composition were estimated using the body composition analyzer (Bodpod). The Bodpod is an Air Displacement Plethysmograph (ADP) which determines body composition fat versus lean by using whole body densitometry. Bodpod's principle is similar to underwater weighing where body mass (weight) is measured. A precise scale and volume are used when sitting inside the Bodpod. Air instead of water is used to measure body volume based on the relationship between pressure and volume. This is the main difference between Bodpod and underwater weighing. When the subjects are seated in the Bodpod, body density is calculated by:  $\text{Density} = \text{Mass}/\text{Volume}$ . Body fat and lean body mass are then calculated once the overall density of the body is determined.

Bodpod is calibrated before any testing. Very small volume changes are produced during a measurement, and the Bodpod measures the pressure response to these changes inside the chamber. The interior volume of the Bodpod chamber is both determined when the chamber is empty and then when the subject is seated. The subject's volume is obtained when subtracting the two volumes. For instance, if the interior volume of the empty chamber is 400 liters, and then is 350 liters when the subject is seated, then the body volume of the subject will be 50 liters.

Two chambers constitute the Bodpod. The unit of the Bodpod is divided by the seat into front (test) and rear (reference) chambers. A common wall is provided by these two chambers where a mounted diaphragm is fluctuating by computer control during testing. The Bodpod door is closed during data collection by a series of electromagnets and a gasket, where the diaphragm oscillates between the two chambers. The volume is increased in one of the chambers and decreased in the other by the same amount. After this volume change, there is a pressure response, and the size of each of the chambers is indicated by the magnitude of the pressure change. The pressure change is rarely noticeable to the subject which is approximately  $\pm 0.5 \text{ cm H}_2\text{O}$ .

The subject is required to breathe normally during the body volume measurement. For the Bodpod, the average lung volume during normal tidal breathing is the relevant measure of the lung volume. Estimation of the effect of skin surface area also takes place. In order to obtain final body composition measurement results, the above information is used to correct the body volume measurement. The test lasts for about 3 minutes, where the door of the Bodpod will open and close between two 50-second trials. It is important that the subject wore minimal clothing with a swim cap so that any air pockets within the hair would be compressed (Inliv, 2018).

#### *4.2.5.4 Resting Lactate*

Resting capillary blood samples were taken after the subjects had placed their hand in warm water for 10 minutes. Participants' finger was then pricked with a disposable lancet so that the skin was punctured, and capillary blood would be taken. The lactate analyzer was calibrated before any analysis. After calibration of the instrument, the blood sample were injected into the sample chamber using a calibrated syringe pipette, and the blood lactate concentration of the sample was determined within one minute.

Blood samples collected at the end of each stage established the lactate curve, and the lactate deflection point was identified as the first increase from baseline in the work/blood lactate curve.

#### *4.2.5.5 Lactate Profile & Incremental Ramp Test*

A Lactate Threshold (LT) test consisted of a graded exercise protocol followed by a  $\dot{V}O_2$  max test. LT test was comprised of 5-minute stages, starting at 7 km/hr, and then the speed was increasing by 0.5 km/hr at every stage. However, at some subjects the speed was adjusted according to their fitness levels and started at a higher speed. At the last minute of each test, expired air samples, HR, and RPE were recorded. Then the participants thumb was pricked in order to take capillary blood sample. At the end of each stage when the blood sample was collected it was then placed in the analyzer to print the lactate value. The lactate values established the lactate curve and the lactate deflection point was identified as the first increase from baseline in the blood/lactate curve. The lactate deflection point was then confirmed as the LT if the collected lactate values kept increasing. The test was then switched to a ramp test, where the speed was increasing by 1 km/hr until exhaustion. When the subject was close to the point of exercise intolerance, serial 1-minute expired gas samples were collected in Douglas bags, for  $\dot{V}O_2$  peak determination.

#### *4.2.5.6 Constant Speed Tests*

Based on the maximal speed that the subject achieved at the incremental ramp test, the Constant Load Tests were individualised to each subject. These tests were designed in such way in order to elicit exhaustion within 3 to 15 minutes (Moritani et al., 1981). The tests commenced with a 5.5 km/hr brisk walk for 6 minutes and then the treadmill would accelerate to the pre-programmed speed for the particular trial. Two stopwatches were used to record the trial's duration, in case of error or malfunction, until the individual would reach exhaustion. Serial expired gas samples were collected in Douglas bags (60 s collection) when the subject was considered to be approximately 2 minutes from exercise intolerance, so that the  $\dot{V}O_2$  peak could be established. The  $\dot{V}O_2$  peak was confirmed as  $\dot{V}O_2$  max when there was no difference in the  $\dot{V}O_2$  peak attained with increases in constant-speed tests (+/- 200 ml). Four Constant Load Tests were required for every participant at each phase, at different velocities so that the CV and D' were attained whilst conforming to Standard Error of Estimates (SEE) before repeating the tests in the second phase in a randomised order.

#### *4.2.6 Data and Statistical Analysis*

A hyperbolic exponential decay relationship between time to exhaustion and speed was used in order to plot each series of constant speed test. The parameters CV (intercept) and D' (slope) were characterized by the speed over time relationship from these tests. These



parameters were estimated using least-squares linear regression of the linear Velocity/time - 1 relationship (i.e.  $V = \left(\frac{D'(km)}{Time(s)}\right) + CV$ ), using OriginPro 7.0 (OriginLab Corporation, Northampton, MA, USA). By estimating SSE, the acceptable limits for CV and D' were of  $\leq 2\%$  and  $\leq 10\%$  respectively. If these criteria were not met, then the speed would be repeated. In order to compare the two phases (BC/PLA) for both CV and D', a paired sample t-test was used. Statistical significance was set at  $p \leq 0.05$ . Data were taken as mean +/- standard deviation.

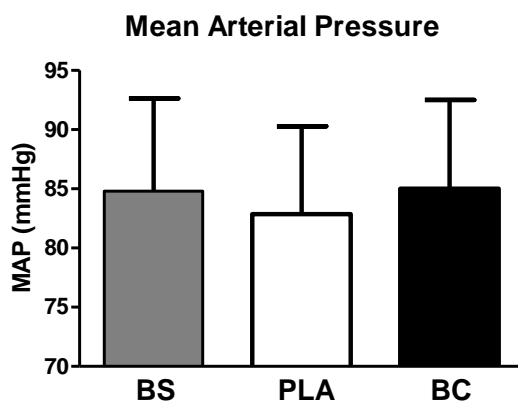
From the IET, both  $\dot{V}O_2$  max and HR max were compared with all the trials of the Constant Load Tests in both BC and PLA by using a one-way ANOVA test. Whether any differences occurred for any of the participants, then that would be identified by a Scheffe Post-Hoc test. RE and Duration from the time trials were tested for repeated measures by using the ANOVA General Linear Model.

### 4.3 Results

Participants were all young, healthy, recreationally active males which is confirmed by the results shown in Table 2. The obtained values indicate that the physiological maximum values were obtained commensurate with age, BMI is on the border between normal weight and overweight (which tends to make trained males appear slightly too heavy if they carry muscle, for example a subject had a BMI of 25.44 but only 10.81% body fat, and fitness of ~50 mL/kg/min is close to the average fitness level one would expect from young adult males that recreationally exercise on a regular, but not very frequent, basis.

Table 3: Subject Characteristics (n=7)	
Age (years)	26 ± 6.6
Height (m)	1.76 ± 0.06
Body mass (kg)	79.25 ± 8.45
$\dot{V}O_2$ max (l/min)	3.96 ± 0.40
$\dot{V}O_2$ max (ml/kg/min)	49.98 ± 2.07
HR max (bpm)	190.86 ± 7.76
Body Fat (%)	10.81 ± 3.56
Fat Weight (kg)	8.5 ± 2.55
Lean Weight (kg)	70.73 ± 8.58
Systolic Blood Pressure (mmHg)	124.14 ± 65.14
Diastolic Blood Pressure (mmHg)	8.65 ± 7.9
Mean Arterial Pressure (mmHg)	84.81 ± 7.83
Body Mass Index (kg/m <sup>2</sup> )	25.44 ± 1.26

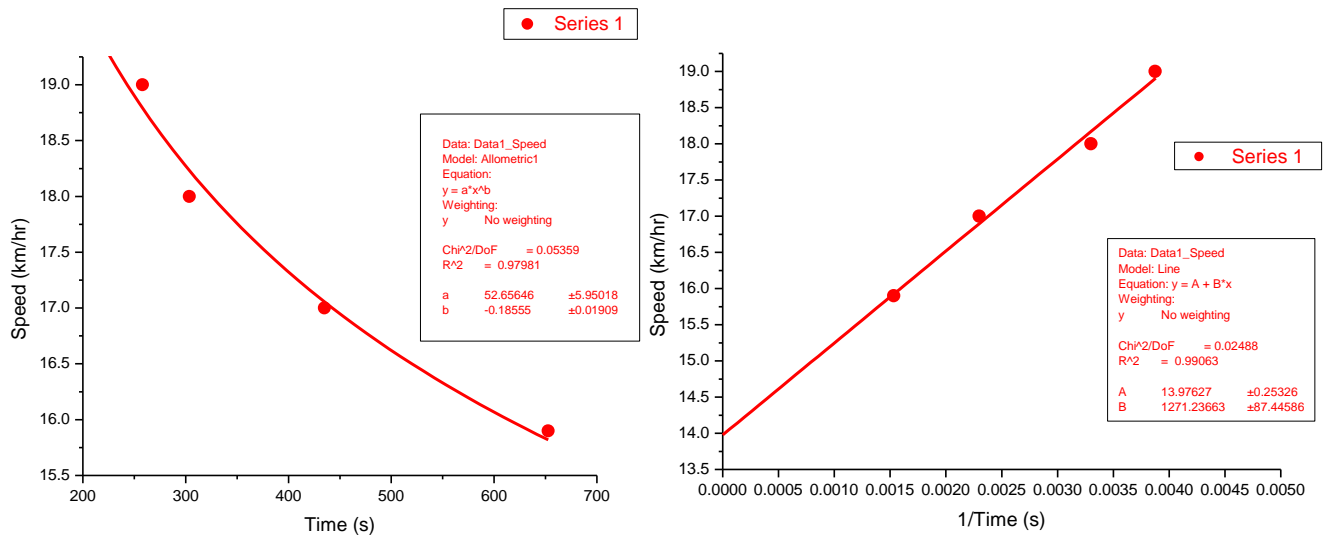
*Table 3: Subject characteristics shown as mean ± standard deviation. Mean values were given by the baseline/Incremental Exercise Test (IET).*



*Figure 12: Mean Arterial Pressure (MAP) during Baseline (BS), Placebo (PLA) and Blackcurrant (BC) conditions. MAP further confirms that the subjects were healthy. There was no significant difference between all three conditions for MAP ( $p=0.844$ ). This suggests that BC extract supplementation did not affect MAP compared to PLA.*

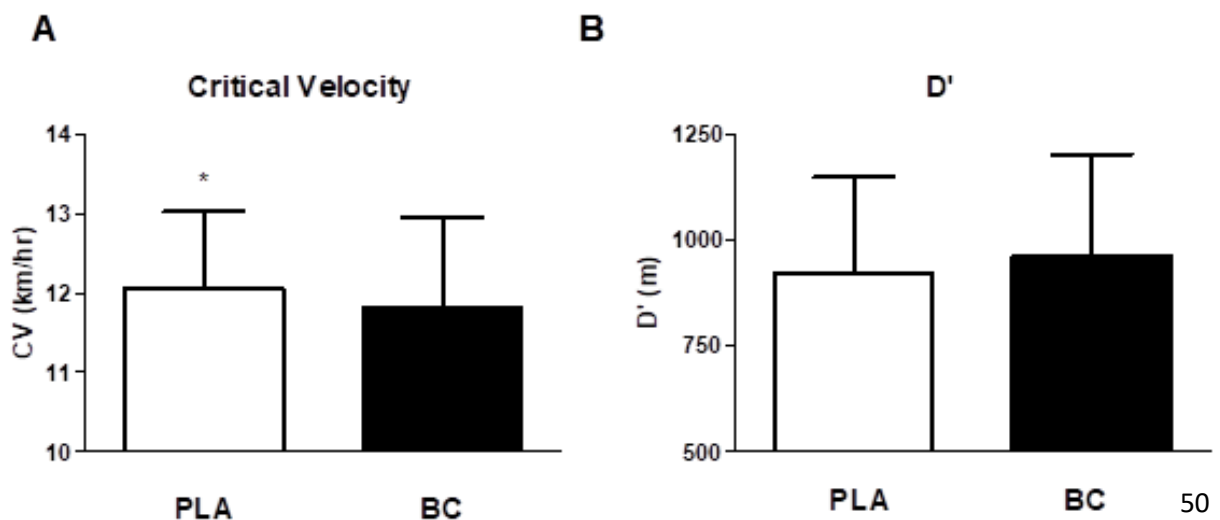
### 4.3.1 CV&D'

After the four constant speed tests, Velocity-time and velocity-1/time relationships were plotted. Figure 13 shows a sample from a subject X. The  $v-1/t$  relationship was used in order to determine the values for Critical Velocity (CV) and Distance Covered D' (determined from the Y-intercept and from the gradient of line respectively).

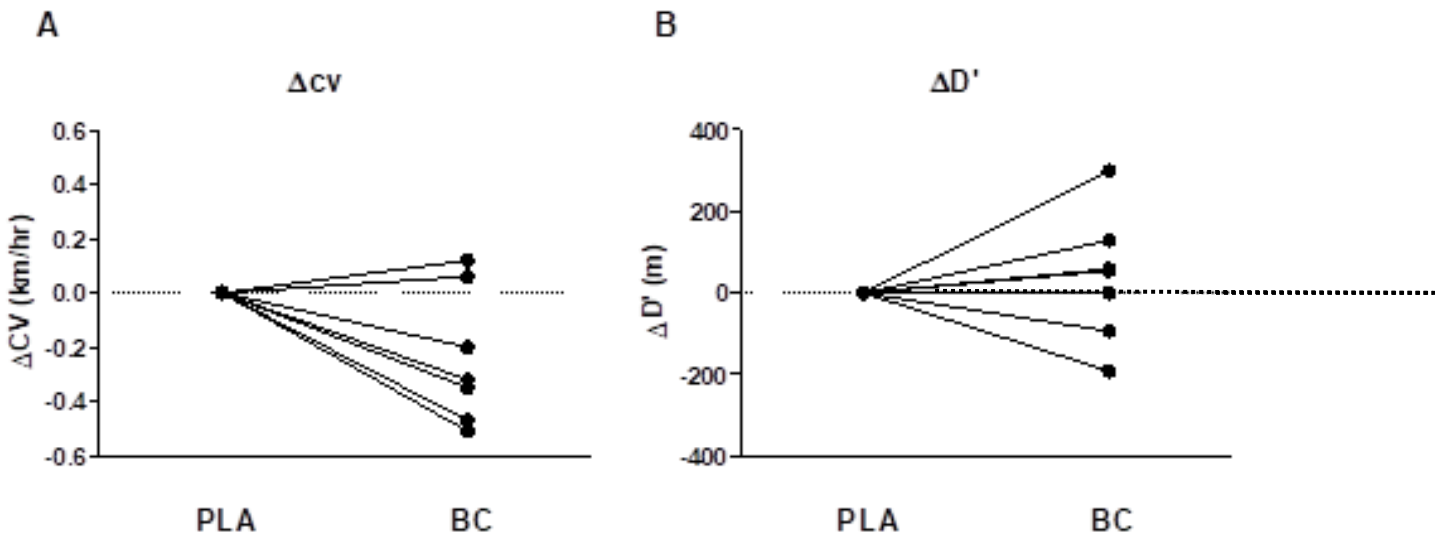


**Figure 13:** Shows a result from a typical example of the V-t and V-1/t relationship respectively. Mean CV and D' was  $13.97 \pm 0.25$  km/hr and  $1271.23 \pm 87.44$  m respectively for this subject X.

Both CV and D' were compared under BC and PLA conditions to examine whether there was any difference. Mean value for CV during BC and PLA was  $11.81 \pm 1.14$  km/hr and  $12.05 \pm 0.98$  km/hr respectively. Mean value for D' during BC and PLA was  $960.18 \pm 234.11$  m and  $923.56 \pm 224.78$  m. Paired-samples t-test showed significant difference over the BC and PLA for CV ( $p=0.043$ , 95% CI = -0.46 and -0.01) but no significant difference for D' ( $p=0.561$ , 95% CI = -109.05 and 182.28). This indicates that BC supplementation did not cause any change to the speed-tolerable duration relationship versus placebo on average.



*Figure 14: Box-plots of mean Critical Velocity (CV) (A) and Distance Covered (D') (B) between Placebo (PLA) and Blackcurrant (BC) conditions. There was a significant difference between PLA and BC for CV but not for D'.*



*Figure 15: Shows the difference of Critical Velocity (CV) (A) and Distance Covered (D') (B) between Placebo (PLA) and Blackcurrant (BC). Shows how much CV and D' changed between the two conditions. The majority of the subjects decreased CV under BC reaching statistical significance towards PLA as shown in Figure 15A.*

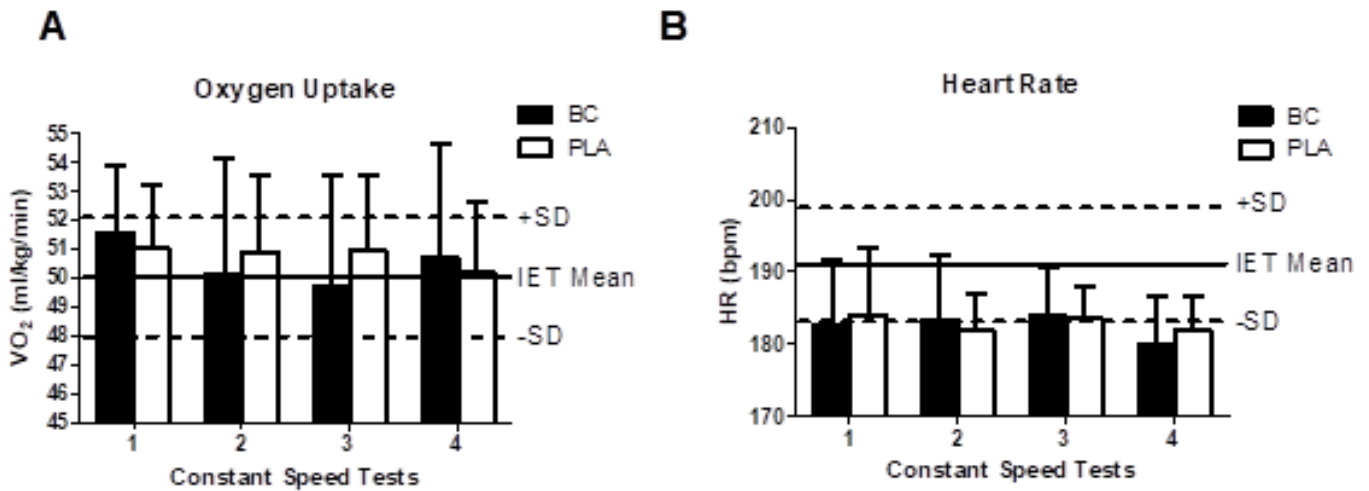
The mean absolute difference of BC-PLA values is displayed in table 4 below where CV was statistically different ( $p < 0.05$ ) between BC and PLA which is further confirmed from the 95% CI were zero is not included. CV was faster under PLA by approximately 2 km/hr. However, D' was higher by 3.81 m under BC supplementation, without reaching significance ( $p > 0.05$ ) since the 95% CI includes zero.

Table 4: Mean absolute BC-PLA differences and analysis from paired samples t-test				
n=7	Absolute difference	95% Confidence Intervals		Significance p=
		(lower)	(upper)	
CV BC-PLA	-1.99	-0.466	-0.103	0.043
D' BC-PLA	3.81	-109.05	182.28	0.561

*Table 4: Mean absolute BC-PLA differences and analysis for CV and D' from paired samples t-test.*

### 4.3.2 $\dot{V}O_2$ max and HR max from Time Trials (TT)

Comparison of  $\dot{V}O_2$  max and HR max was then applied between the IET and all trials from the Constant Load Tests. As confirmed by the one-way ANOVA test, there was no significant difference for both  $\dot{V}O_2$  max ( $p=0.981$ ) and HR max ( $p=0.370$ ). This indicates that each time trial was executed to exhaustion, which therefore for the purpose of this study made them valid for calculating CV,  $D'$ , and the speed-tolerable duration relationship.

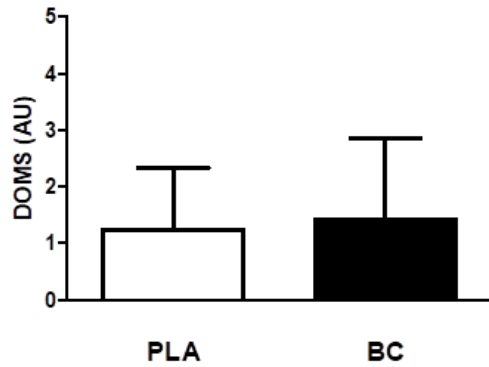


*Figure 16: Box-plots of maximal  $O_2$  uptake ( $\dot{V}O_2$  max) and maximal Heart Rate (HR max) over the two conditions of constant speed tests. No significant difference for both  $\dot{V}O_2$  max and HR max under Blackcurrant (BC) and Placebo (PLA). In these figures, the full line and the two dashed lines, indicate the mean  $\pm$  SD for the  $\dot{V}O_2$  max and HR max from the IET test, against which the  $\dot{V}O_2$  and HR are obtained from the time trials, and were then compared.*

### 4.3.3 Delayed Onset of Muscle Soreness (DOMS) from TT

DOMS was averaged from Time trials 1, 2 and 3. Paired samples t-test was used to identify any differences between BC and PLA groups. There was no significant difference between BC and PLA for DOMS ( $p=0.611$ , 95% CI = -0.67 and 1.05). This suggests that the muscle mechanical impact or fatigue experienced by the subjects did not differ while on BC versus PLA.

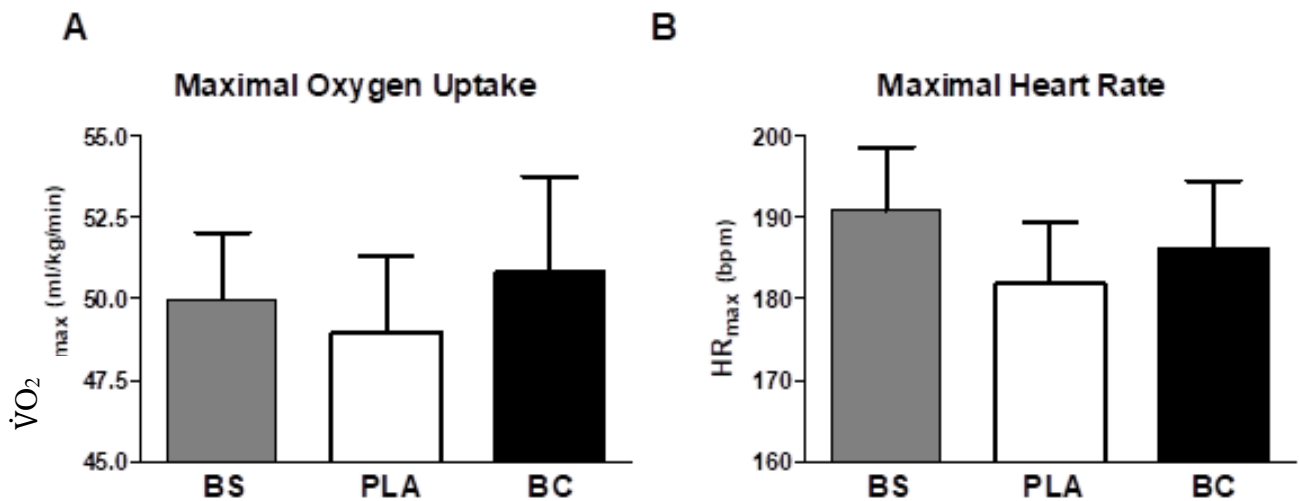
**Average Delayed Onset of Muscle Soreness**



*Figure 17: Box-plots of average Delayed Onset of Muscle Soreness (DOMS) between the two groups. No significant difference was found between Blackcurrant (BC) and Placebo (PLA).*

#### 4.3.4 $\dot{V}O_2$ and HR max from IET during Lactate Threshold (LT)

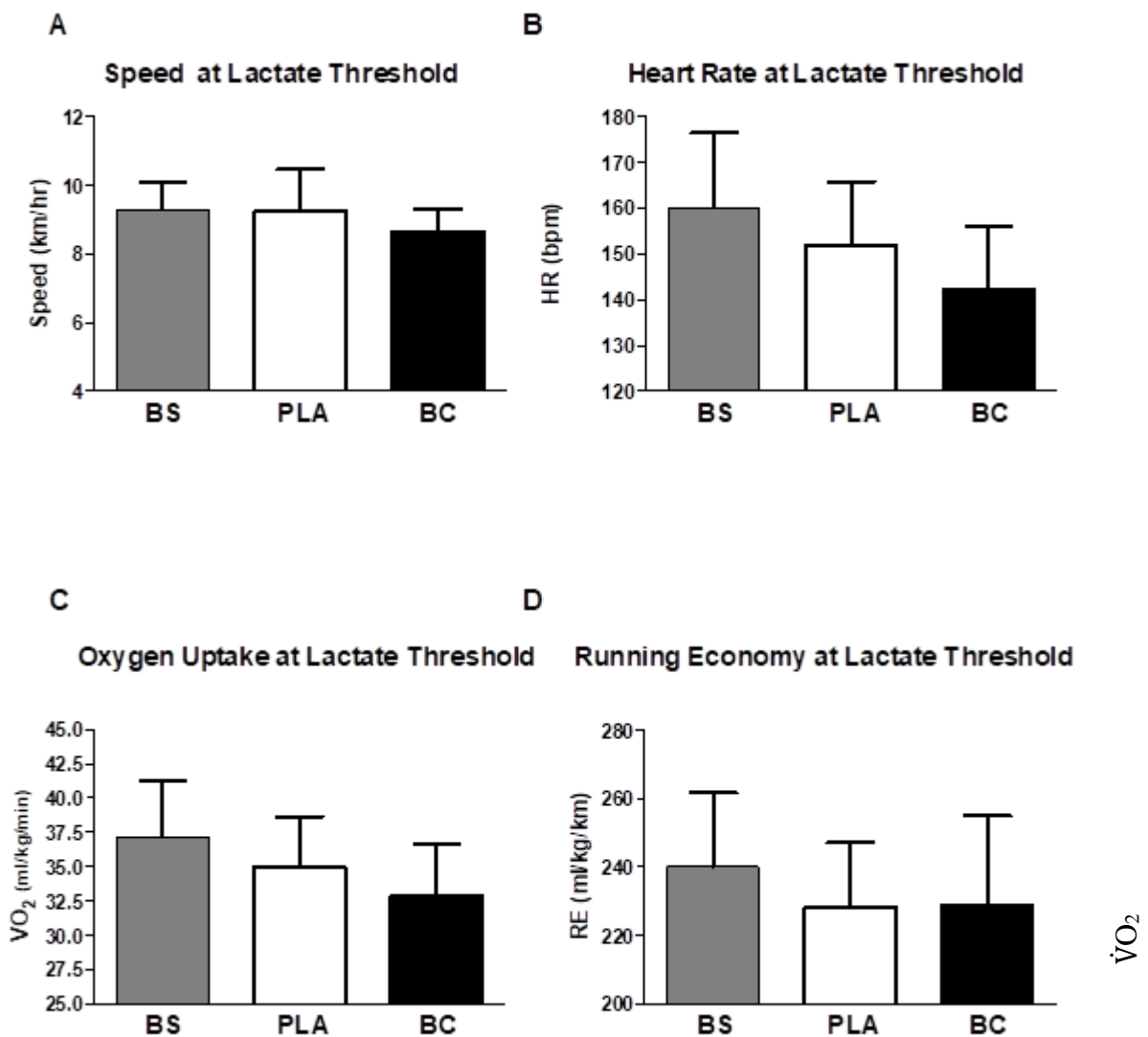
Results from the IET were compared between the three conditions (BS, PLA and BC) to identify if there were any differences in any of the parameters. There were no differences in either  $\dot{V}O_2$  max ( $p=0.402$ ) or HR max ( $p=0.129$ ) between the three conditions as shown in Figure 18. This indicates that BC extract did not affect  $\dot{V}O_2$  max or HR max in comparison with PLA on average.



*Figure 18: Box-plots of mean maximal  $O_2$  uptake ( $\dot{V}O_2$  max) (A) and maximal Heart Rate (HR max) (B) during the Incremental Exercise Test (IET) under the three different conditions. No differences were found between the three groups as confirmed by the one-way ANOVA.*

#### 4.3.5 Speed, HR, $\dot{V}O_2$ and RE from IET during LT

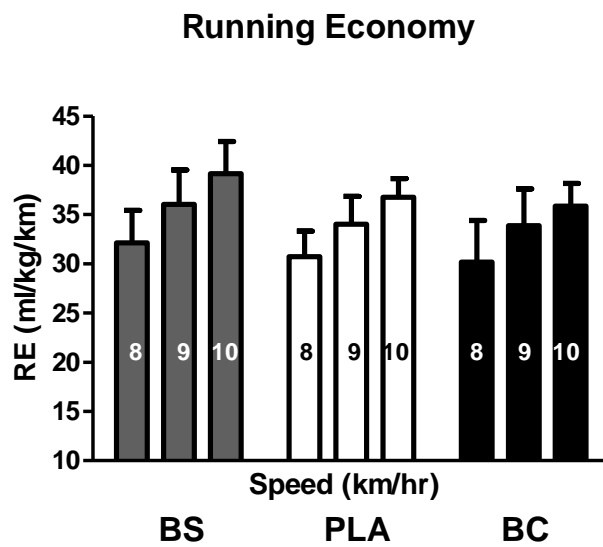
During the IET, Speed, HR,  $\dot{V}O_2$  and RE at LT were compared during BS, PLA and BC. There was no significant difference for Speed at LT ( $p=0.381$ ), HR at LT ( $p=0.103$ ),  $\dot{V}O_2$  at LT ( $p=0.146$ ) or RE at LT ( $p=0.551$ ), as shown in figure 19. This suggests that Speed, HR,  $\dot{V}O_2$  and RE at LT did not change with BC supplementation on average, versus PLA. There is a trend for a lower HR with BC as shown in figure 19B by 6.83 bpm compared to PLA and 11.25 bpm compared to BS. This trend is also observed in figure 19C for  $\dot{V}O_2$  with BC by 6.42 ml/kg/min compared to PLA and 11.48 ml/kg/min compared to BS. However, both parameters did not reach statistical significance since the p-value was not less than 0.05.



*Figure 19: Box-plots of mean Speed (A), Heart Rate (HR) (B), Oxygen uptake ( $\dot{V}O_2$ ) (C) and Running Economy (RE) (D) at Lactate Threshold (LT) during the Incremental Exercise Test (IET). No significant difference between the groups for any of the parameters.*



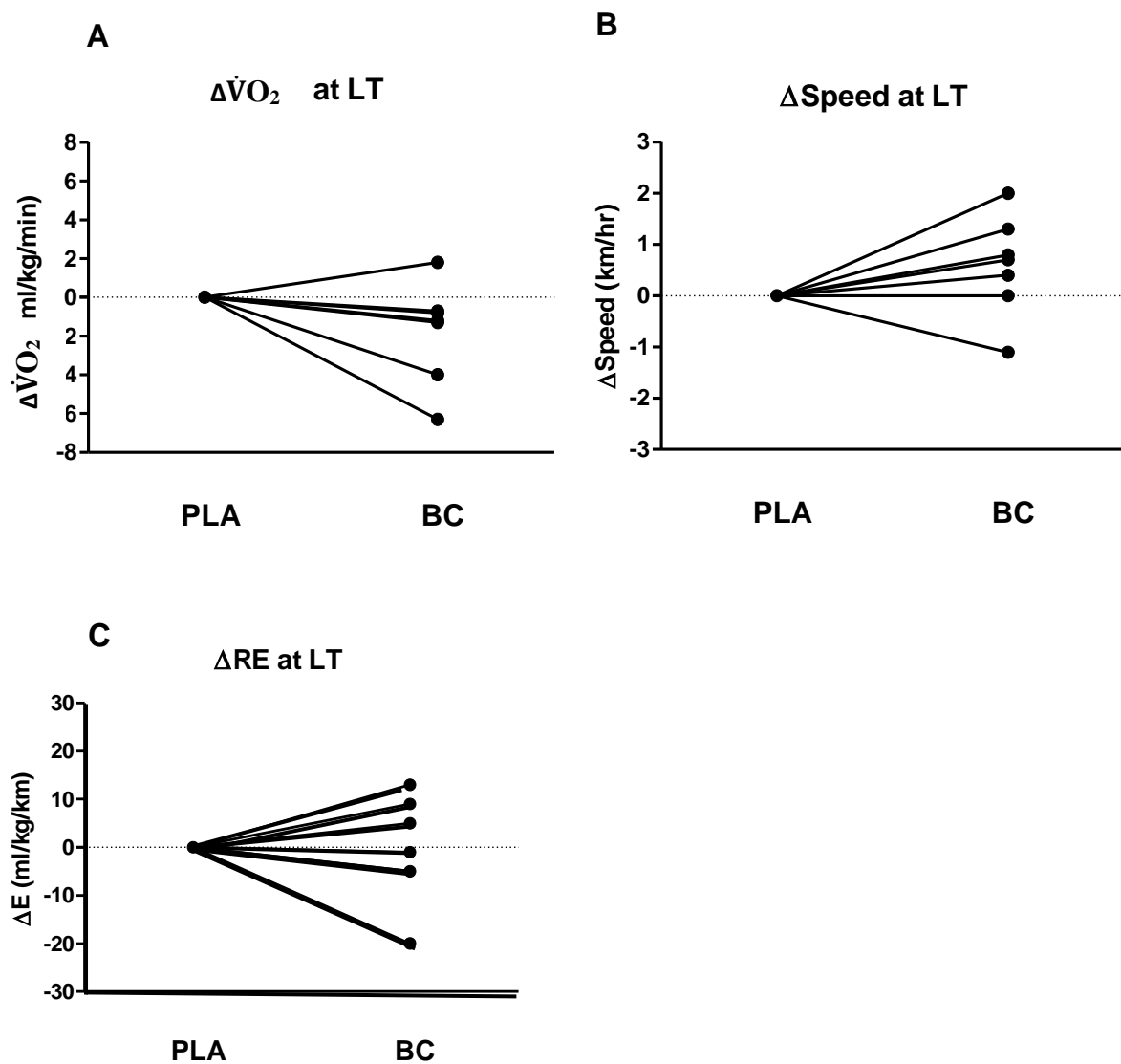
RE was further tested by repeated measures with the general linear model at speed 8, 9 and 10 km/hr for all subjects.



*Figure 20: Shows the bar-plots of the means at three different speeds during Lactate Threshold (LT) for the three different groups. There was no significant difference between any of the speeds at LT between the groups. This shows that BC did not affect RE at any speed, on average, compared to PLA.*

#### 4.3.6 $\Delta$ for parameters that determine aerobic capacity

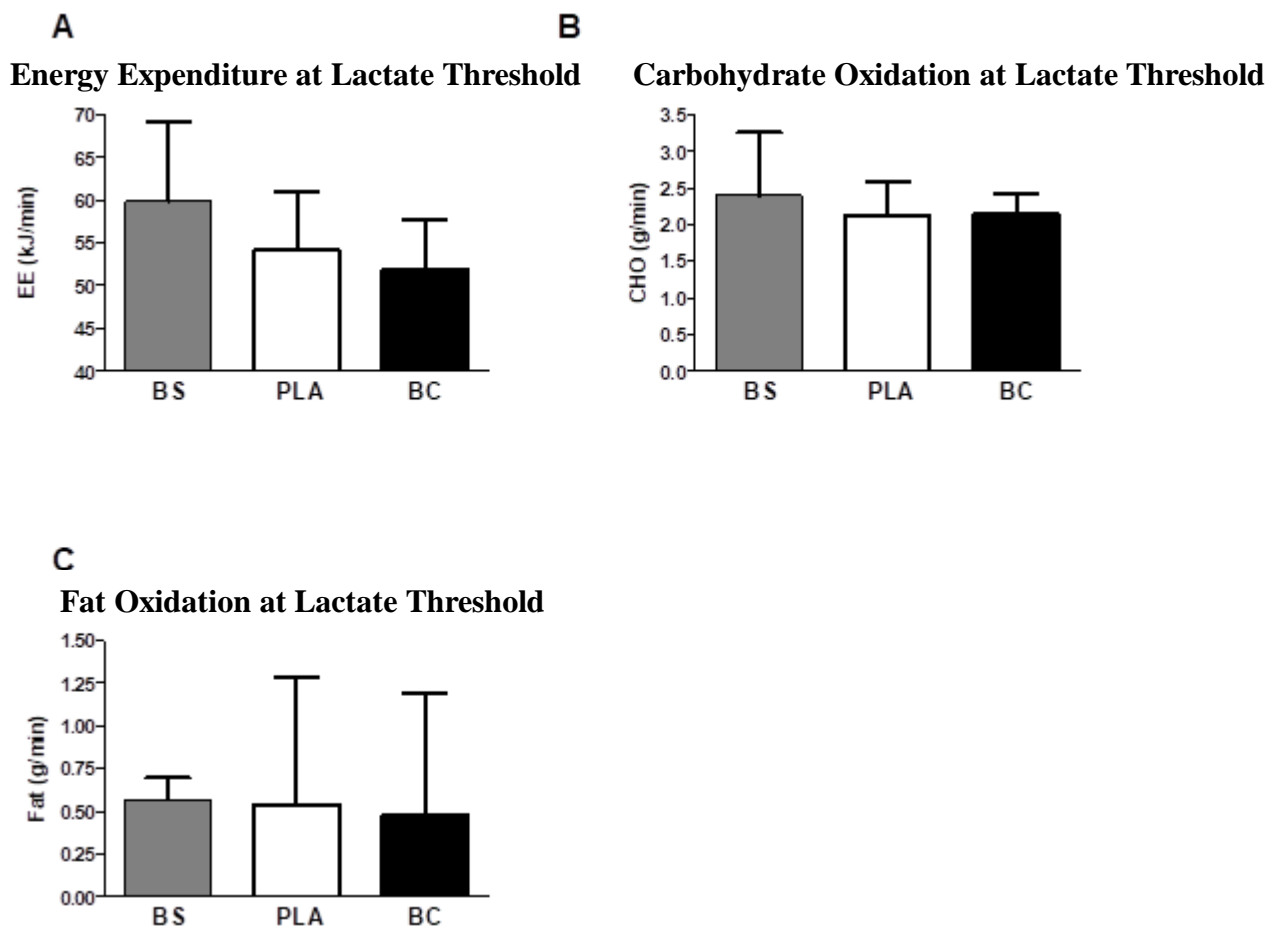
The difference ( $\Delta$ ) of the three parameters which determine the aerobic capacity of an individual ( $\dot{V}O_2$  max, Speed and RE at LT) was further analysed. Five subjects increased their Speed, one showed no change and one decreased their Speed under BC supplementation. Five out of seven subjects decreased their  $\dot{V}O_2$  with the BC extract. Three subjects increased, and four subjects decreased their RE under BC supplementation.



*Figure 21: The difference of maximal Oxygen Uptake ( $\dot{V}O_2$  max) (A), Speed (B) and Running Economy (RE) (C) at Lactate Threshold (LT) between Placebo (PLA) and Blackcurrant (BC). In figure 21A the majority of the subjects seem to decrease  $\dot{V}O_2$  max under BC condition whereas in figure 21B and 21C subjects increased their speed and RE at LT with BC.*

#### 4.3.7 Oxidation parameters during LT

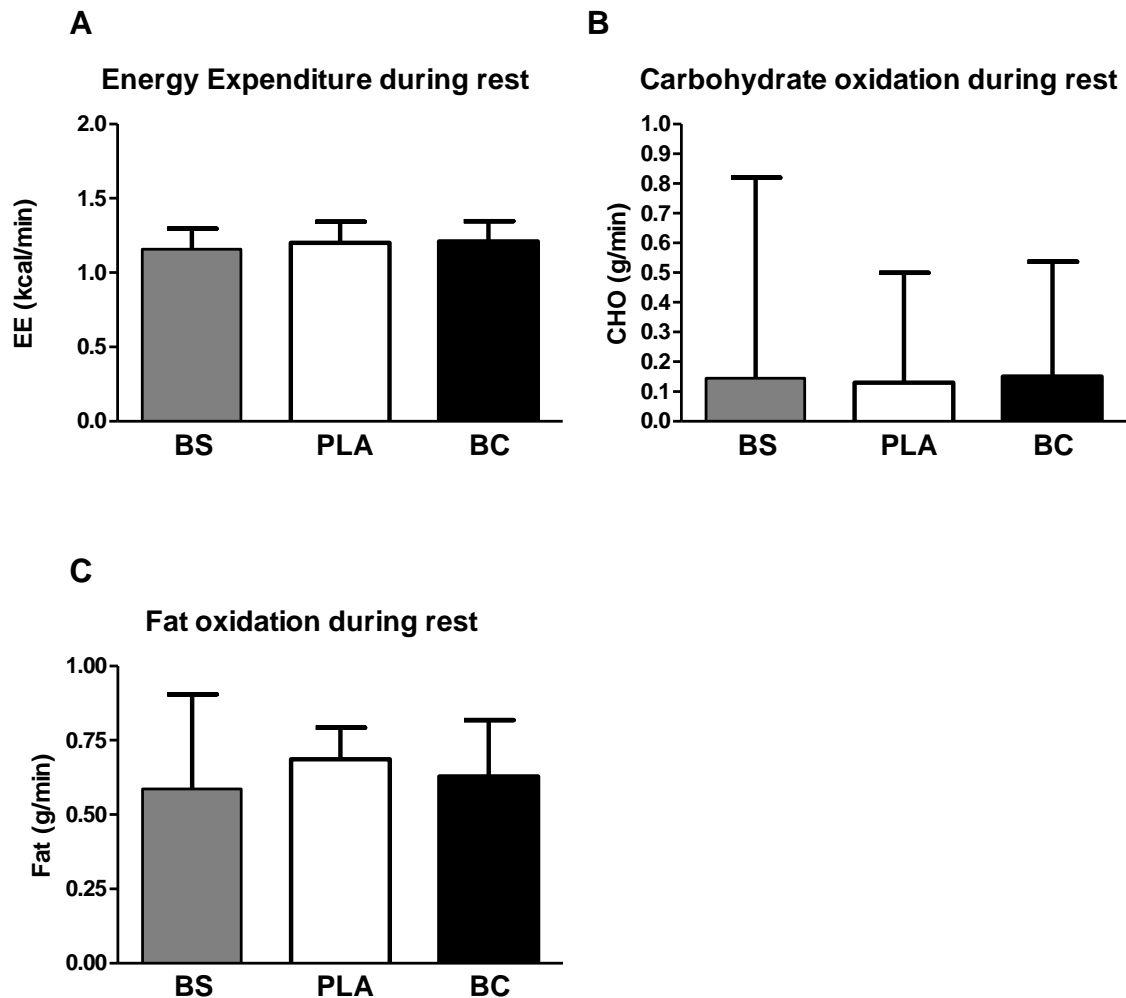
Oxidation parameters involving EE, CHO and fat oxidation at LT were also examined for any differences between the three groups, shown in Figure 22. It was found that BC caused no differences in EE ( $p=0.151$ ), CHO oxidation ( $p=0.619$ ) and Fat oxidation ( $p=0.177$ ) when compared to BS and PLA which suggests that BC extract does not affect EE, CHO and Fat oxidation at LT when compared to PLA. A trend towards a lower EE can be observed in figure 22A with BC by 4.05 kJ/min without reaching statistical significance. Compared to BS, EE was again reduced with BC by 13.2 kJ/min, but no statistical significance was confirmed.



*Figure 22: Bar-plots of mean Energy Expenditure (EE) (A), Carbohydrate Oxidation (CHO) (B) and Fat (C) oxidation under Baseline (BS), Placebo (PLA) and Blackcurrant (BC) at Lactate Threshold (LT). Figure 22A shows no significant difference between the three groups ( $p=0.151$ ). Similarly figure 22B and 22C show no significant difference between the groups at LT ( $p=0.619$  and  $p=0.177$  respectively).*

#### 4.3.8 Oxidation parameters during rest

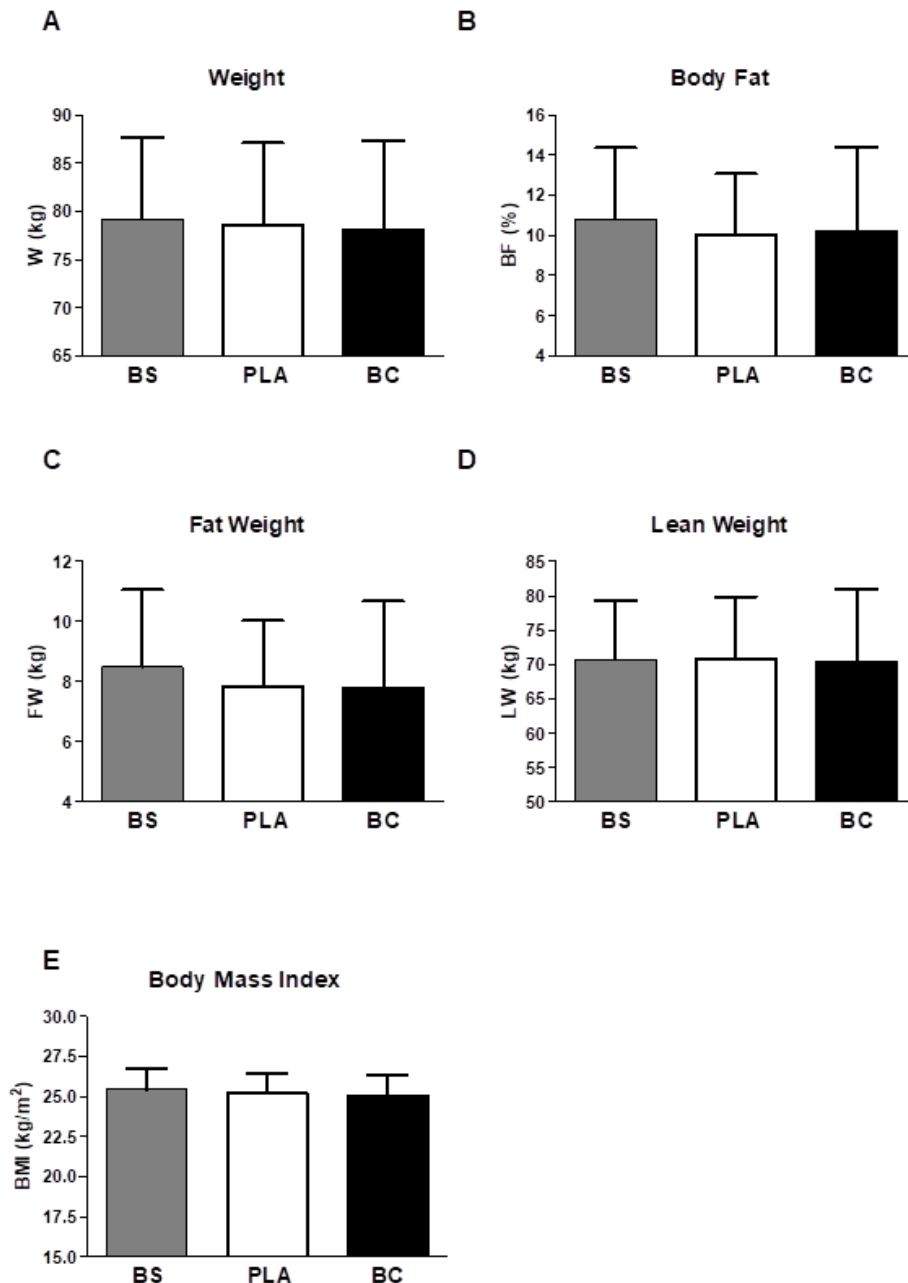
During rest, EE, CHO and Fat oxidation were analysed for any differences within BS, PLA and BC. As confirmed by the one-way ANOVA test there were no significant differences for EE ( $p=0.753$ ), CHO oxidation ( $p=0.744$ ) and Fat oxidation ( $p=0.705$ ) during rest. This indicates that BC did not affect EE, CHO and Fat oxidation in comparison to BS and PLA on average.



*Figure 23: Bar-plots of mean Energy Expenditure (EE) (A), Carbohydrate Oxidation (CHO) (B) and Fat oxidation (C) during rest. No significant differences were found as confirmed from the p-values ( $p=0.753$ ,  $p=0.744$  and  $p=0.705$  respectively).*

#### 4.3.9 Bodpod Measurements

Results from Bodpod measurement including weight, body fat, fat weight, lean weight and body mass index were also examined for any differences between BS, PLA and BC. There were no significant differences between weight ( $p=0.967$ ), body fat ( $p=0.916$ ), fat weight ( $p=0.842$ ), lean weight ( $p=0.992$ ) and Body mass index (BMI) ( $p=0.823$ ) between the groups. This indicates that BC did not affect weight, body fat, fat weight, lean weight and BMI when compared to PLA on average.



*Figure 24: Bar-plots of mean Weight (W) (A), Body Fat (BF) (B), Fat Weight (FW) (C), Lean Weight (LW) (D) and Body Mass Index (BMI) (E) between Baseline (BS), Placebo (PLA) and Blackcurrant (BC). No significant difference was found for any of the parameters between the three groups.*

The mean absolute BC-PLA difference and analysis are demonstrated for all variables from Paired-samples t-test in table 5 below. As shown from the table, there was no significant difference for any of the parameters since the p value was higher than 0.05 and this is also confirmed from the 95% CIs where all intervals include zero. The values that were increased with the intervention were  $\dot{V}O_2$  max by 3.74 ml/kg/min, HR max by 2.30 bpm, RE at LT by 0.24 ml/kg/km, CHO at LT by 0.46, EE at rest by 0.82 kcal/min, CHO at rest by 13.3 g/min and Body fat by 1.54% although they did not reach statistical significance. In contrast, the values that decreased with the intervention were Speed at LT by 6.32 km/hr, HR at LT by 6.83 bpm,  $\dot{V}O_2$  at LT by 6.42 ml/kg/min, EE at LT by 4.05 kJ/min, Fat oxidation at LT by 12.1 g/min, Fat oxidation at Rest by 8.8 g/min, Weight by 0.57 kg, Fat weight by 0.32 kg, Lean Weight by 0.82 kg and BMI by 0.67 kg/m<sup>2</sup>.

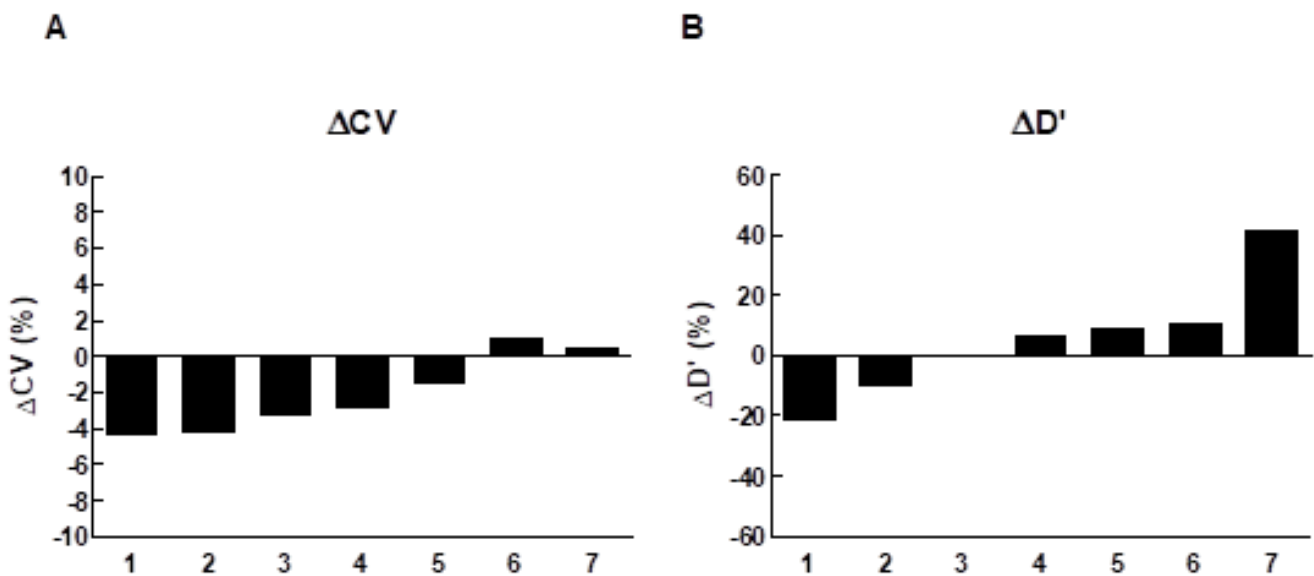
**Table 5: Mean absolute BC-PLA difference and analysis for all variables from one-way ANOVA test**

	Absolute Difference (BC-PLA)	95% Confidence Intervals		Significance p=
		(lower)	(upper)	
$\dot{V}O_2$ max (ml/kg/min)	3.74	-0.573	4.222	0.112
HR max (bpm)	2.30	-2.546	11.117	0.176
Speed at LT (km/hr)	-6.32	-1.494	3.226	0.166
HR at LT (bpm)	-6.83	-21.804	2.376	0.097
$\dot{V}O_2$ at LT (ml/kg/min)	-6.42	-5.049	0.895	0.138
RE at LT (ml/kg/km)	0.24	-0.585	10.728	0.895
EE at LT (kJ/min)	-4.05	-5.873	1.476	0.194
CHO at LT (g/min)	0.46	-0.240	0.260	0.925
Fat ox at LT (g/min)	-12.1	-0.168	0.371	0.169
EE rest (kcal/min)	0.82	-0.0262	0.462	0.524
CHO rest (g/min)	13.3	-0.206	0.606	0.274
Fat ox rest (g/min)	-8.8	-0.024	0.103	0.493
Weight (kg)	-0.573	-1.936	1.036	0.487
Body Fat (%)	1.54	-1.588	1.902	0.833
Fat Weight (kg)	-0.32	-1.341	1.284	0.959
Lean Weight (kg)	-0.82	-2.811	1.639	0.543
BMI (kg/m <sup>2</sup> )	-0.67	-0.691	0.354	0.460

*Table 5: Mean absolute BC-PLA difference and analysis for all physiological variables from Paired-samples t-test.*

#### 4.3.10 Individual Analysis

From a statistical point of view the results showed no effect on  $D'$ . However, on closer look on individual analysis, 60% of subjects showed positive results towards BC, although this did not reach statistical significance. When analysing CV, this trend was much smaller. This suggests that under the particular circumstances, BC may result in a positive effect in certain individuals.



*Figure 25: Bar-plots of the difference of Critical Velocity (CV) (A) and Distance Covered ( $D'$ ) (B) for each subject. As shown on figure 25A, 5 out of 7 subjects decreased their CV with Blackcurrant (BC) whereas 2 of them showed an increase. Figure 25B shows that 4 out of 7 subjects increased their distance covered from 6% up to 41%, one of the subjects showed no change, and 2 of the subjects decreased their  $D'$  without reaching statistical significance. Positive values show which subjects improved their CV or  $D'$  whereas negative values indicate those who decreased their CV or  $D'$ .*



## 4.4 Discussion

### 4.4.1 Speed-tolerable duration, CV and D'

Based on the protocol followed, the effects of NZBC on CV and speed-tolerable duration relationship during running were tested. Four Constant Load Tests were performed in a randomized order, based on the velocity, which was achieved during the IET, where the subjects ran until exercise intolerance. In order to examine the effects of BC on the CV concept, this was required for the subsequent analysis, as set out by the aims of this study. According to the results that have been obtained and analysed, it is suggested that BC has no effect on CV and D'. There was a significant difference between PLA and BC for CV although it was not biological significant as the improvement with PLA was almost only 2 km/hr. This is the first study to be conducted which examined the effects of NZBC on the concept of CV and speed-tolerable duration relationship during running. However, when looking into individual improvements instead of average only, it was found that 60% of the subjects showed improvement on D', although it did not reach statistical significance. Four out of seven subjects showed improvements from 6% up to 41% on their distance covered. However, when analysing CV, this trend was much smaller where only 2 out of 7 subjects showed improvements. This trend would in such case be in agreement with Willems et al., 2014 who found that repeated sprint distance in a high-intensity intermittent running test was improved by 10.8%.

An important pre-requisite of this study was that during exercise at velocities above CV,  $\dot{V}O_2$  max was always reached in spite of different speeds and durations tested within the constant speed tests, indicating that CV was appropriately assessed, and all the tests were exhaustive. This is consistent with a study by Hill and Ferguson (1999) who showed that at speeds above CV,  $\dot{V}O_2$  max was always reached, and it was achieved in shorter duration when the velocities were higher. The fact that HR values were below the -SD (see Figure 16B) is because the Constant Speed Tests are different kind of tests than the IET. The IET is used to test the maximum values whereas the Constant Speed Tests are not designed to stress HR as much as IET. Constant Speed tests are not assessed by HR although the requirement for properly assessing CV is achieving  $\dot{V}O_2$  max since  $\dot{V}O_2$  max is given by the Fick equation  $\dot{V}O_2 \text{ max} = SV \times HR \times (a-v O_2 \text{ difference})$ , where HR is only a component of the equation.

The peak speed that was achieved during the IET provided an estimation of the speed that was to be used for the first Constant Speed Test. For the rest of the Constant Load Tests, the speeds that were used, were set in order to elicit exhaustion within 3 to 15 minutes. The tolerable duration is unlikely to be determined by the same mechanisms of fatigue if any of these tests are out with this period. If that is the case, then the tolerable duration does no longer rely on the rate of D' depletion. Tests with a tolerable duration of less than 2 minutes might be determined by the ability of the muscle to generate the necessary force to sustain the exercise. Motivation, nutrition and thermoregulation are more likely the factors that determine tests that last longer than 15 minutes (Poole et al., 1990).

### 4.4.2 $\dot{V}O_2$ max, LT, and RE

When the results from the IET were compared between BS, PLA and BC conditions, there was no effect from BC in any of the parameters that were examined including  $\dot{V}O_2$  max, HR, Speed at LT, HR at LT,  $\dot{V}O_2$  at LT and RE at LT. The  $\dot{V}O_2$  max was slightly higher with BC

extract but not significantly. A study by Willems et al. (2014), also found that there were no alterations in physiological responses including HR,  $\dot{V}O_2$  max and La.

In our study there was no differences in (blood or plasma) La and this disagrees with another study by Cook et al. (2015), where plasma La was higher with NZBC extract immediately following the TT where 7-day intake of BC supplementation was used. Willems et al. (2014), examined the effects of 7 days Sujon BC powder intake on the blood La curve and aerobic capacity of trained triathletes and it was found that Sujon BC resulted in a complete shift of the lactate curve and the power at OBLA was 6% higher with Sujon BC with 85% of the triathletes showing increase and no effect on HR and  $\dot{V}O_2$ . There was 14% decrease in La at  $\dot{V}O_2$  max in 77% of subjects. In the study of Cook et al. (2015), the subjects that participated were healthy men, recruited from triathlon and local cycling clubs. They should be involved in sports for more than 3 years, and they should typically perform cycling exercise 8-10 hours a week in order to participate in the study. They also had a personal best time of less than 30 minutes for a 16.1 km cycling time-trial. The study of Cook et al. (2015), had 14 subjects comparing to our study which had 7 subjects. In our study, the subjects that participated were coming from different sports background and not just one particular. The dosage of supplement was the same (300 mg/day CurraNZ containing 105 mg anthocyanin) in both studies.

In another study by Willems et al. (2014), Sujon BC decreased Total Peripheral Resistance by 16% in 78% of the participants with no changes in BP or HR. In our study there was no change in MAP or HR which is in agreement with the study of Willems et al. (2014). In the study of Willems et al. (2014), 13 trained triathletes participated with more than 3 years of triathlon experience. Supplementation involved consumption of Sujon BC powder (6g/day, 138.6 mg of anthocyanin) for 7 days. This study involved both male and female triathletes. A commercially available blackcurrant juice containing about 3-4 mg anthocyanin per dose, was used for the PLA.

In both Cook et al. (2015) and Willems et al. (2014) studies, the subjects might have had more experience than our subjects. In the study of Willems et al. (2014), female subjects were also involved, and participants consumed greater dosage of anthocyanins compared to our study. Placebo was also blackcurrant juice, although had a small amount of anthocyanins, it could still have an impact on the effects that were shown in the study. Psychology could play a major role, as the subjects from those studies could be more familiarised with the protocols compared to our subjects who perform exercise to exhaustion very rarely.

Running Economy did not improve on average in our study with BC extract which is in agreement with Willems et al. (2014), who found that cycling economy did not change between CurraNZ and PLA at 45%, 55% and 65%  $\dot{V}O_2$  max.

However, on a study which looked at the dose effects of NZBC on substrate oxidation and physiological responses during prolonged cycling (Cook et al., 2017), it was found that 7-day intake of NZBC extract showed a dose-dependent effect on augmenting fat oxidation during 120-min cycling at 65%  $\dot{V}O_2$  max in endurance-trained male cyclists. The participants completed 4 separate 120-min cycling bouts at 65%  $\dot{V}O_2$  max after ingesting no dose, or one of 3 doses (300, 600 or 900 mg/day) of CurraNZ for 7 days. In this study the physiological responses remained unchanged including HR,  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , plasma La and glucose. There was a 21.5 and 24.1% increase in mean fat oxidation rates and absolute increases of 0.11 and 0.10

g/min for 600 and 900 mg/day NZBC intake showing moderate-to-large effects. These doses are twice and three times the one that Cook et al. (2015), used in another study with 300 mg/day showing no difference in average fat oxidation. There was also no effect of BC in any dose for cycling economy. This study suggests that no adverse physiological effects can occur with high-dose intake of NZBC in trained cyclists.

#### 4.4.3 Oxidation parameters during LT

There were no differences between BS, PLA and BC conditions for EE, CHO oxidation and Fat oxidation at LT. However, Fat oxidation was the lowest with BC extract ( $\mu=0.47$ ) at LT compared to BC ( $\mu=0.57$ ) and PLA ( $\mu=0.54$ ). These results agree with the study of Willems et al. (2014), where again it was found that fat and CHO oxidation did not change between CurraNZ and PLA at 45%, 55% and 65%  $\dot{V}O_2$  max. However, in another study by Cook et al. (2015), NZCB extract increased fat oxidation at 65%  $\dot{V}O_2$  max by 27%. In this study, subjects took 7 days supplementation and then performed 30 min of cycling (3x10 min at 45, 55 and 65%  $\dot{V}O_2$  max), followed by a 16.1 km TT with lactate sampling during a 20-min passive recovery. The 16.1 km cycling TT performance was improved, and fat oxidation was increased during moderate intensity cycling. In that study, the observed alterations in substrate utilization occurred after a standardized absolute carbohydrate intake 2 hours prior to the event, with no alterations in glucose or hypoglycaemia and the authors suggest that BC might have additional effects on lipid metabolism.

#### 4.4.4 Oxidation parameters during rest

During rest, EE, CHO and fat oxidation were analysed to obtain any potential improvements with the BC extract. However, there were no significant differences found between conditions for any of these parameters that were examined. These findings suggest that BC extract as an antioxidant causes no alterations in physiological parameters. There was no study up to this day, to examine the effects of NZCB extract as a supplement on resting metabolism. However, it is suggested that chronic BC extract intake in C57BL/6J mice elevated mRNA of genes involved with Energy Expenditure in a study by Benn et al. (2014). Also, another study by Tsuda et al. (2005), observed that treatment of rat adipocytes caused upregulation of 633 genes including genes involved in lipid metabolism and signal transduction. Therefore, it was suggested that a combination of many pathways that act in cooperation may increase whole-body fat oxidation, upregulate genes for proteins involved in fat oxidation, transport of fatty acids into mitochondria, improved NO availability and therefore, increased peripheral blood flow.

#### 4.4.5 Delayed Onset of Muscle Soreness (DOMS)

BC extract had no effect on DOMS, as there no difference was found between the TT. That contradicts another study where subjects performed a typing work for 30 minutes, they consumed 50 g anthocyanin which resulted in significantly higher total and oxygenated haemoglobin whereas in the PLA group, there was a significant stiffening of the trapezius muscle (Matsumoto et al., 2004). However, typing performance with the BC intake did not improve. There is no research conducted up to date that assessed DOMS with BC intervention with similar biomechanical properties to exercise.

#### 4.4.6 Body Composition Estimations

There were no changes in body weight, body fat, fat weight, lean weight and BMI between BC and PLA. When looking on individual basis, 4 out of 7 subjects had reduced their body fat with the BC extract however that did not reach statistical significance. The subjects' BMI was found quite high and most of the subjects were in the 'overweight' category. However, the NHS (and other health authorities) threshold is 25.0 kg/m<sup>2</sup> which works for large populations, although can be different for sports people. Athletic populations tend to have greater muscle mass compared to non-athletic, which typically artificially elevate BMI since muscle weighs more than fat and adipose tissue of the same volume.

Our results are in agreement with those of Yarahmadi et al. (2014), a study that looked at the effect of anthocyanin supplementation on body composition, exercise performance and muscle damage indices in athletes. In this study, soft lean mass, total body water and body fat percentage did not change significantly in the anthocyanin group after intervention however,  $\dot{V}O_2$  max increased significantly in the anthocyanin group. There was also no significant decrease in the Creatine Kinase or Lactate Dehydrogenase between anthocyanin and placebo groups. This study was a double-blinded clinical trial which involved participation of 54 female (22) and male (32) athletes with athletic history of at least 3 years. Participants consumed 100 mg/day anthocyanin or placebo for 6 weeks.

In the majority of the previous studies, there were not significant differences reported in body composition within antioxidant consumers. A clinical trial by Knab et al. (2011), studied the effects of quercetin (a plant flavonol from the flavonoid group of polyphenols), in which the participants were divided into 3 groups of 500 mg daily quercetin, 1000 mg daily quercetin and PLA. Similar to our study their results showed no significant differences between the groups in terms of BMI or any other body composition indices.

The variation in results across studies may be attributed to the differences in the duration and intensity of exercise, as well as the exercise mode, training status of participants and the use of a variety of direct or indirect measures of performance.

#### 4.4.7 Experimental and biological variability

According to Atkinson and Nevill (1998) reproducibility of measurements "is considered to be the amount of measurement error that is deemed acceptable for the practical and effective usage of a measurement tool". This means that variation would always exist between measurements however, a set limit should be acceptable. There is always going to be variability in both the participants and the testing, in other words biological and mechanical variation, which could possibly explain the results of this study. In sport and exercise sciences the criteria that are in common use as an acceptable level of measurement error are correlations of more than 0.9 and sample mean coefficients of variation of less than 10%. In this study, when the standard error (SE) was greater than 2% for CV and 10% for D' or the coefficient of variation was too high then the results were rejected, and the test had to be repeated. However, few tests had to be accepted due to various factors, specifically time constraints. Another important factor was that, the subjects were required to perform at least 11 tests during this period of study and if they had to repeat some tests could possibly lead them to withdrawal from study due to psychological factors and lack of motivation.

Both biological and mechanical variations can be controlled to an extent. As an example, the protocol was designed to limit the variations in performance that may be caused by the variations in the circadian rhythm, where the testing took place at the same time of day. Another common variation might be within the individual and the equipment. Mechanical error for example the equipment calibration, human error and mechanical variation and biological error such as daily internal fluctuations, mental state, and physiology, may cause  $\pm 2\%$  variation which is considered acceptable regardless of an arbitrary decision (Hopkins, 2000). Further studies need to assess the validity of the CV concept (Atkinson and Nevill, 1998).

#### 4.4.8 Individual analysis

The participants that were recruited had different athletic profiles and training status. This heterogeneity may have contributed to the variability in the effects and the lack of significant and clear trends in the results. The subjects came from backgrounds that included weightlifters, cross fitters, martial arts athletes, climbers, sprinters and endurance runners. Considering the individual analysis, the participants that improved their distance covered ( $D'$ ) were the weightlifters, cross fitters, climbers and sprinters and not the endurance trained athletes. Also, the endurance trained athletes did not show any improvements in any of the parameters that were measured in this study. The non-endurance trained subjects were the ones who showed improvements in their  $D'$  whereas the endurance trained subjects slightly improved their CV with BC supplementation. The  $\dot{V}O_2$  max for the endurance runners may have been expected to be larger than the rest of the participants, however that was not the case. Some of the weightlifters, cross fitters and sprinters were well trained individuals and probably this is why their  $\dot{V}O_2$  max was a bit higher than expected. In addition, some of the participants were more familiarized with the treadmill running compared to others, as they use some aerobic exercise in their daily exercise routine whereas others only perform weightlifting exercises. Endurance athletes have a high CV relative to  $\dot{V}O_2$  max and have only modest  $D'$  whereas sprint trained athletes tend to have a low CV relative to  $\dot{V}O_2$  max and much higher  $D'$  which was the case in some of the participants in this study.

#### 4.4.9 Better athletes, better results?

The psychological profile and training experience play an important role at higher standard runners, which makes their results better when comparing with the recreational/ club level individuals. It is hard to measure psychological aspects including determination and motivation. However, these are extremely important when maximal intensity exercise is considered, where a higher standard athlete with faster  $\dot{V}O_2$  kinetics,  $\dot{V}O_2$  and PCr recovery will probably have an advantage (Jones, 2002). Due to the fact that the tests in this study were exhaustive, may have negatively affected some of the subjects' motivation. Better athletes with high and consistent motivation will prevent potential reduction in motivation. Recruitment of runners was much more difficult in this kind of study which requires multiple exhaustive tests, due to the training interruptions, number of athletes, availability to test, time in season, athlete's schedule and coach and athlete cooperation.

Additionally, various supplements have recently been examined whether their ergogenic properties of performance-enhancing supplementation would be affected by the training status of an athlete. Beneficial effects were reported for trained subjects only during morning testing with caffeine supplementation. However, beta-alanine resulted on beneficial ergogenic effects on both trained and untrained cyclists for lower-body Wingate tests. A

study by Godwin et al (2017) examined the effects of NZBC after 7-day intake of 600 mg/day NZBC and showed that supplementation of NZBC was more effective for repeated sprint performance in trained football players compared to recreationally active males. Fatigue index was reduced by 12% for all participants combined, however the number of non-responders was high. However, the dietary intake was not controlled throughout the study therefore, the amount of anthocyanin and polyphenols could probably be already higher before supplementation. This study suggested that trained, or higher standard athletes would be more benefited from NZBC supplementation compared to recreationally active athletes.

#### 4.4.10 Standardization of program

In order to standardize the pre-test conditions, participants were advised to avoid any caffeine, food and training before their trials and IET. These conditions can only control testing to a certain degree. Different external variables including food intake, injury, stress, weekend hard training, illness and sleep may explain differences in the time to exhaustion. There is no direct type of measurement of all the psychological aspects which may feature to variations in performance. In spite of the lack of psychological control, efforts including pre-programmed treadmill, the same time of day testing ( $\pm 1$  hour) and the instructions to avoid certain things before testing, were combined in an attempt to better standardize the procedures.

#### 4.4.11 Strengths/Limitations of Study

Few strengths and limitations of this study might be the reason for the results that were caused or not caused by the BC supplementation. A change in  $\dot{V}O_2$  max or subjects' V-t relationship might be caused from an increase in tolerable duration at the given velocities causing some of the subjects to probably have a training response which could potentially explain some individual improvements in this study. However, all participants were healthy, young and recreationally active therefore a training effect is unlikely to occur. A training effect is likely to be of importance for those who are not accustomed on exercise. Whether these tests would show improvements is dependent on the subjects' fitness and training status. Fitness levels can change if someone stops exercising and fluctuations can happen. However, a wash-out period of 14 days was required in this study because supplementation of a substance was involved.

With the current experiments, no trends towards effects of BC were detected, hence we decided to not continue with the current protocols and studies. If in contrast we had detected a possible effect, we would have continued the experiment. Different population of participants, for instance runners, that are more motivated or better able to engage their own gait should be tested to observe any effects from the supplement.

The loading of BC extract might have had an impact on the results of this study. The majority of studies had a 7-day supplementation of BC extract of 105 mg anthocyanin according to manufacturer guidelines. In this study, subjects were instructed to take a supplement every day 30 minutes before their testing which included 105 mg anthocyanin or PLA. Tests could have been cancelled or rescheduled because of the subject's daily schedule or responsibilities; therefore the subjects were given more supplements until their next test. However, the loading did not have an impact as shown from the results. Different loading or a more sustained loading might have caused different results.

Another concept to consider when discussing about supplementation includes the responders versus non-responders. This concept has not been studied regarding BC extract, but it has been assessed on training and performance. The phenomenon of responders to training has been proven in different pieces of research. Individuals have a wide range of responses to different interventions rather than a similar response. Therefore, they can be divided into high-responders, low-responders and non-responders. A study by Mann et al. (2014), suggested that genetic influences contribute to individual variations in some training responses. Also, training which is prescribed by relative intensity and duration may be more successful in causing a similar homeostatic stress between individuals than other methods. Different exercise stimulus may be caused and contribute to individual responses in the adaptive responses of the training program. Recovery may also play an important role which may differ between individuals due to factors including sleep, training status, habitual physical activity and psychological stress. Nutritional factors can also contribute to individual variations in training responses including the timing and composition of dietary intake. It is suggested that endogenous and exogenous substrate availability may adjust the transcriptional and translational response to an exercise bout. The messenger RNA expression of genes involved in lipid metabolism and protein degradation is attenuated by the ingestion of carbohydrate or carbohydrate-protein mixture, respectively. Mann et al. (2014) suggested that the non-genetic determinants of training responses including sleep, nutritional status and homeostatic stress of a training session need a better understanding in order to achieve optimal training effect.

Table 6: Correlates of the gains in the $\dot{V}O_2$ max in the Heritage Family Study	
Parameter	Percentage variance explained
Baseline $\dot{V}O_2$ max	2%
Age	3%
Sex	3%
Weight	3%
Ethnicity	3%
Achived vs targeted training $\Delta W$	6%
Technical error & daily $\Delta s$	20%
Heritability	47%

*Table 6: Summarizing the correlates of the gains in the  $\dot{V}O_2$  max in the Heritage Family Study by Sarzynski, Ghosh and Bouchard (2016). Shows that the estimated heritability of the trainability of the  $\dot{V}O_2$  max is 47% compared to other factors that contribute in a much less percentage.*

In addition, the tests were performed in lab-based exercise protocols where the environmental conditions are very controlled. A 1% gradient was set for the treadmill for outdoor running replication, in order to match the energetic cost of the treadmill exercise with that of the outdoor running. It is not known whether hill gradients or weather conditions could have influenced the speed-tolerable duration relationship. The physiological demands of sports may not have been required by the lab-based exercise protocol that was performed in this study, where random and multiple changes in direction and speed would occur in the field.

#### 4.4.12 Future Work

There are some routes from our results which could be examined in further research. An example of a different test that the subjects would have been advantaged from would be to suggest a race that would confine with CV, a context of a type of exercise that the concept of CV is valid. This test could be a time trial for example a 10 km race where exhaustion should be achieved within 20-30 mins and the concept of CV is still valid. And then, supplement the subjects with different doses of BC, for example separate them into different dosage groups of 300, 600 and 900 mg/day of BC, to see whether any of the doses would have an effect on CV. This could provide further evidence whether greater dosage would influence CV rather than a lower dosage. However, it is possible that high doses of BC may be contraindicated and counterproductive. If the mechanism is that BC stimulates endothelial function either directly or indirectly by an antioxidant action, then a high dose could theoretically lead to global peripheral vasodilation, and this could reduce blood flow rather than increase it, if all or the majority of blood vessels are relaxing and vasodilating. This would mean that not only are muscle blood vessels dilating, but also vessels perfusing other organs, skin, etc, and so the total effect would be too much vasodilation and a resultant reduction in blood flow everywhere, since there may not be sufficient blood to enhance flow everywhere. This is only a speculation, therefore further studies are required to clarify whether higher dosages would be effective or harmful.

Future studies should examine dosing strategies which focus on clarification of the frequency, optimal dose and duration of intake. This is because the mechanisms that are causing the effects are complex due to the availability of various metabolites in the blood which would potentially lead to affection of cell function and performance enhancement. Physiological effects particularly for the amount, type and duration of polyphenol intake may explain the performance enhancement, hence the effects of this supplement should examine the exercise performance and physiological responses. Any effects that are produced by BC should be further investigated whether they occur because of the anthocyanin content only or whether a combination of substances act synergistically. Up to date there is no evidence to suggest that these benefits that studies claim to find are caused due to anthocyanins only. Consideration needs also to be taken regarding the participant's training status, sex and substrate oxidation that may affect the observed lactate responses. Field based protocols should also be applied in order to replicate the conditions of different sports or even match conditions. The factors that affect metabolism, absorption, distribution and elimination of anthocyanins needs further investigation. It was mentioned that anthocyanins have low bioavailability and poor stability at serum or cellular pH, and their absorption reaches peak levels in the blood within 1 to 2 hours where their elimination is completed after 48 hours (Matsumoto et al., 2004). Due to their low concentration in vivo and their poor stability, anthocyanins can be challenging to detect and precisely quantify. Therefore, longitudinal research with long follow ups are needed to evaluate the effects of anthocyanins.



## **5.0 General Discussion and Conclusions**

Critical Velocity (CV) and distance covered before exhaustion (D') can be used to tailor exercise prescription to improve an athlete's training and performance. This is especially true for higher intensity exercise programs exceeding the runner's CV, such as high intensity interval training or competitions. For recreational runners, knowledge of CV and D' can help prescribe an exercise program that improves aerobic capacity. In competitive performance, CV can be used as a pacing strategy whereby the athlete's pace can be modified so that the distance is completed in the most efficient way and the pace is steadily spread across the race. It can also serve as a parameter that records an athlete's improvements after periods of training. Hence, it is critical that knowledge of CV and D' is of high fidelity and investigating methods to improve these two performance characteristics is warranted.

The aim of the study was to test the reproducibility of the CV concept over two series of exhaustive tests on controls (non-runners) and runners and examine the effects of the supplement New Zealand Blackcurrant on CV and speed-tolerable duration relationship during running. The distinction between controls and established runners is of particular interest since measurement of the CV does not take into account voluntary fatigue, i.e. a runner's tendency to stop even though he/she can sustain the exercise for longer periods. Established runners and elite athletes are not considerably influenced by voluntary fatigue as they tend to be better prepared to mentally engage and drive their performance to the individual's true exercise limits. The hypothesis to be validated by the reproducibility study is that CV should be reproducible for the established runners while this would not be the case for non-runners who may be more susceptible to voluntary fatigue. Hence, usage of the concept of CV could potentially be more applicable to established runners and high standard athletes in prescribing exercise regimens and formulating running strategies. Moreover, future reproducibility studies should utilize professional or elite athletes even though it is more challenging to recruit such athletes.

The subjects performed Incremental Exercise Tests followed by four Constant Load Tests over two series at different speeds in a randomized order. These tests suffice in capturing the V-t relationship from which CV and D' can be extracted. Measurements showed that for non-runners, there was a significant test-retest difference for both CV and D'; in other words, they were not reproducible. However, for the runners, there was no significant difference for CV and D', indicating they were reproducible. The results thus showed that CV as well as D' were reproducible for the runners but not for the controls. This suggests that runners are more consistent in their trials and can produce almost the same values of CV and D' during repetitive measurements due to the fact that they are more able to engage their own gait and sustain the exercise more consistently compared to the controls, who might not be that familiar performing exercise to exhaustion. Nevertheless, average values for CV and D' were not significantly different between the runners and the controls. This might be because the runners in our study were not professionals or elite level athletes but recreational/club level athletes. Therefore, higher standard athletes or elite level athletes may differ with respect to their results compared to the recreational/club level athletes. However, recruitment of this kind of athletes is more difficult. Another finding of the study was that the  $\dot{V}O_2$  max and HR max were always achieved at velocities above CV showing that at each constant load test the exercise intensity was high enough for  $\dot{V}O_2$  max attainment. Therefore, it was confirmed that the exercises led to fatigue which is a prerequisite for establishing the value of CV.

The second part of the study examined the effects of New Zealand Blackcurrant on CV and speed-tolerable duration relationship during running and resting metabolism. CV and D' were calculated by having seven participants perform at least 4-time trials on the treadmill either on BC or PLA supplementation, where they ran to exhaustion. Based on average values the results showed that BC as an ergogenic aid has an insignificant effect on CV and D' or any of the other physiological parameters measured. However, analysing the results on an individual basis, it was found that 4 out of 7 subjects improved their D' by 6% up to 41% with the BC extract. This raises the possibility that BC may improve performance for some individuals based on factors that require further and more detailed investigation.

Another finding of this part of the study was that BC did not cause any changes to resting metabolism, including EE, CHO and Fat oxidation. BC did not affect the oxidation parameters (EE, CHO and Fat oxidation) during LT either. This suggests that when taking PLA or BC the subject would burn the same amount of CHO and Fat and use the same EE on average. Body composition measurements including body weight, body fat, fat weight, lean weight and BMI were not affected by BC or PLA. This indicates that the subjects did not lose any weight, body fat, fat weight or did not gain any lean weight and the value of their BMI did not change on average. DOMS also did not change by BC or PLA on average, suggesting that the level of soreness for each subject depending on how sore they claimed that they were feeling prior to each trial did not change with PLA or BLA supplementation. However, body fat was reduced with BC extract on individual basis in 4 out of 7 subjects, although not statistical significance was reached. The majority of the subjects' BMI was found quite high, placing them in the 'overweight' category. For large populations, the NHS threshold is 25.0 kg/m<sup>2</sup>, however this may be different for sports individuals that often carry more muscle than fat or adipose tissue compared to normal controls. As such, muscle mass is greater in the athletic compared to the non-athletic population, hence the BMI can be higher.

BC extract did not change any of the aerobic capacity parameters including  $\dot{V}O_2$  max, HR max, Speed at LT, HR at LT,  $\dot{V}O_2$  at LT and RE at LT when compared to BS or PLA conditions. In the BC extract condition, the  $\dot{V}O_2$  max was slightly higher however, yet it did not reach statistical significance. RE was also increased in the majority of the subjects on individual basis, but again without any statistical significance. As there were no differences in the aerobic capacity parameters between PLA and intervention, this suggests that the performance of the subjects was not improved. Even though the study suggests minimal effects by supplementation with BC, several factors that can alter the results should be the focus of future studies, especially considering that on an individual basis noteworthy effects were identified.

The psychological profile and training experience play a significant role in the athletic performance. Higher standard athletes have different psychological profiles along with training experience when compared to recreational or club level individuals. When maximal intensity exercise is involved, psychological aspects such as motivation and determination play a key role, however these parameters are hard to measure. Higher standard athletes with elevated  $\dot{V}O_2$ ,  $\dot{V}O_2$  kinetics, and PCr recovery will be more consistent with high motivation. This type of athletes will restrict any reductions in motivation or negative thoughts. In future studies, a different type of testing could be used such as a race, where the subjects would have advantaged from, where the concept of CV is valid. For example, a time trial such as a 10 km race, achieving exhaustion within 20-30 mins. This is a fixed distance that could also mimic the demands of competitive race. A more focused study that recruits established,

trained runners exclusively would be prudent, even though it is much more challenging to recruit such subjects when multiple exhaustion tests are involved.

Different doses of BC extract could also be investigated, for instance 300, 600 or 900 mg/day which would provide evidence if larger dosage would alter the CV concept. Such study could also address the possibility of high dosage counterproductive effects (that were mentioned in the discussion section 4.4.12), including the reduction of blood flow which would be caused by global peripheral vasodilation. Dosing strategies should also be tested in order to clarify the frequency, the optimal dose and the duration of intake. Further investigation with the BC extract should aim to clarify whether anthocyanin alone or a combination of substances are causing those effects. No evidence exists, up to date, to suggest that anthocyanin is the only reason for the resulting changes or effects. Factors affecting absorption, metabolism, elimination and distribution of anthocyanin needs to be studied further. It is difficult to detect the anthocyanin due to their low bioavailability and poor stability. Peak levels of absorption are reached in blood within 1 to 2 hours of ingestion, and elimination is completed after 48 hours. Detection and quantification of anthocyanin is challenging hence why longitudinal research along with long follow ups are required for evaluation of its effects.

In conclusion, these investigations found first that CV and D' were less reproducible in healthy young adult control subjects than subjects actively engaged in running through participation in a running club (thus, runners versus controls). Secondly, it was found that BC extract had an insignificant effect on CV and D', as well as the oxidation parameters (EE, CHO, Fat oxidation) during rest and at LT. Additionally, BC extract showed no effects on either body composition measurements or any of the physiological parameters that were measured.

## 6.0 References

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