CHAPTER 2

METHODOLOGY
2.0 Methods

2.1 Participants

Twenty-four healthy participants (17 male and 7 female) from the University of Chester volunteered to take part in this study (mean (SD) age 21.4 (2.6) years, height 1.74 (0.10) m and body mass 72.2 (12.3) kg).

All participants were adjudged to be apparently healthy by conforming to the following inclusion criteria:

- Free from disease, illness and injury as assessed by a pre-test questionnaire (Appendix C);
- Resting heart rate <100 bpm, as determined by health screen;
- Blood pressure <170/100 mmHg, as determined by health screen;
- Participants’ habitual physical activity levels were also identified by the pre-test questionnaire.

Exclusion criteria:

- Resting heart rate >100 bpm
- Blood Pressure >170/100 mmHg
- Any illness or injury
- Having had a recent heavy meal and/or been drinking alcohol in the last 24hr.

All participants were asked to abstain from alcohol, caffeine, nicotine, and strenuous physical activity on the day of each test. All participants were given a participant information sheet and asked to complete an informed consent form and a pre-test questionnaire prior to being tested (Appendix A, B, and C, respectively). Participants were asked to attend each of the study’s four trials in an as near to identical state as
possible to the others, with regards to diet, exercise and sleep patterns in the 24hrs prior to testing.

Each participant was tested at a time of day similar to their previous test/s (within 2 hrs) to control for physiological variation caused by circadian rhythms (Reilly, 2007). It was also stipulated to participants that they should avoid eating within a period of three hours before each test.

For this study to go ahead, ethical approval was granted by the ethics committee of the Centre for Exercise and Nutrition Science at the University of Chester.

To ensure participant confidentiality an identification numbering system was introduced, participants were allocated a number between one and eighteen, from there on this was how they were referred to throughout the study.

2.2 Experimental Design

The investigation was designed to assess the validity and repeatability of predicting acceptable estimates of $\sqrt{V_{O2max}}$ from sub-maximal intensities corresponding to the perceptual RPE grades of 9, 11, 13, and 15.

The study followed a repeated-measures design, consisting of four testing sessions per participant on a Woodway PPS55 Sport-I Treadmill (USA). All testing was performed at the Physiology Research Laboratory at the University of Chester. The four trial design (three perceptually guided exercise tests (PGXTs) followed by a single graded exercise test to maximal exertion) allowed for initial familiarisation and preceding habituation to the RPE scale and exercise equipment (such as face mask) under repeated exercise conditions as well as psychological preparation for the maximal GXT.

It was necessary for all participants to perform the GXT as the final exercise test to support the validity of the study. Performing the GXT prior to sub-maximal PGXTs
would have provided participants with prior knowledge and experience of the Borg scale and an entire range of sensational exertion, thus making the production trials easier to judge accurately. This would have compromised the reliability of the study in regards to its aim, because the procedure would be intended for application within a setting where patients or performer’s are unable to exercise to $\dot{V}O_2_{max}$. Therefore, results rely on the notion that the participants have little or no prior knowledge of a maximal test or the Borg RPE 15-point scale, and instead will be able to improve perceptual accuracy with practice.

**Experimental Design schematic:**

18 participants ($n = 18$) volunteered to participate in the study

Participants given the option to attend familiarisation session

Participants undertake 3 PGXTs on Separate 3 occasions (see section 2.6.2)

Participants perform GXT (see section 2.6.2)
2.3 Exercise Testing

Each testing session consisted of a three phase approach, and present at each session was a qualified laboratory technician and first aider:

Phase I: Measurements taken on arrival.
Phase II: Treadmill protocol and data recording.
Phase III: Supervised cool down and observation of participant condition to allow for safe recovery.

2.3.1 Phase I: Measurements taken on arrival.

On arrival at the laboratory the participants were asked to rest on a chair for ten minutes, to ensure that they were in a rested state before taking measurements and commencing exercise. Whilst seated the participants were required to complete a consent form (first session only, as it covered the entire study) and a pre-test questionnaire (see Appendix C). These questionnaires were analysed to check that the participant had refrained from any strenuous activity up to 48 hrs before testing, had not consumed any substances which may have influenced performance (such as nicotine, caffeine or alcohol) in the 24 hrs prior to exercise, and had abstained from a heavy meal in the three hours leading up to testing.

Once the participants were suitably rested, anthropometric measurements of stature and mass were recorded (see section 2.4.1 and 2.4.2, respectively), however stature was only recorded in the initial session. All data was recorded on to a data collection sheet (Appendix E).

Intra-arterial blood pressure (BP) was measured using a sphygmomanometer (Spengler Vaquez Lauby Classic, France) via a hand held continuous wave Doppler (Huntleigh Healthcare Dopplex D900, UK) (see section 2.4.3).
Meteorological readings of room temperature (°C), pressure (mmHg) and humidity (%) were also taken immediately before each exercise test to provide a record of laboratory conditions and by which to calibrate the gas analyser (Oxycon Pro, Germany).

2.3.2 Phase II: Treadmill protocol and measurements.

Following anthropometric measurements participants were fitted with a heart rate monitor (Polar Electro, Kempele, Finland) to measure heart rate consistently throughout the entire duration of the test. Participants were then fitted with a suitably-sized Hans Rudolph face mask which was connected to an online gas analyser (Oxycon Pro, Germany) to collect and record all inspired and expired gases, thus providing O₂ uptake (oxygen consumption) as well as the respiratory exchange ratio (RER) values (see section 2.5.4).

The participant was then guided on to the treadmill to undertake the exercise protocol (treadmill protocol outlined in section 2.6.2).

Measurements taken during treadmill protocol:

Gas analysis – Recorded constantly by online, breath by breath gas analysis (Oxycon Pro, Germany), providing O₂ uptake and RER readings (see section 2.4.5). Written measurements were taken every three seconds as a guide and back-up source.

Heart rate – Measured every three minutes using the Polar Electro (Kempele, Finland) heart rate monitors.

Respiratory Exchange Ratio (RER) – Also, measured at three minute intervals and provided by the Oxycon Pro (Germany).

Time – treadmill protocol was timed and readings were taken at three minute stages during the actual test.

Gradient (%) – Gradient was recorded every three minutes and manually altered during PGXTs by the researcher under instruction by the participant, so to regulate intensity appropriately (see section 2.6.2 for speed/gradient ratios).
Speed (km/h) – Speed was recorded every three minutes, participants manually altered speed to remain within a prescribed RPE level (see section 2.6.2 for speed/gradient ratio).

2.3.3 Phase III: Supervised cool down and participant observation to ensure safe recovery.

On termination of each test a cool down was compulsory, gradient was reduced to 0% and speed was gradually lowered until heart rate dropped below 100 bpm. The participant was provided with a cool glass of water and asked to sip slowly. Once heart rate returned to within a normal range, the participant reported no ill feelings and the supervisor was satisfied with their apparent condition the participant was deemed fit to leave.

2.4 Measurement Procedures

2.4.1 Stature (Height) Measurement

Participant’s height was measured to the nearest tenth of a 0.1 cm using a wall stadiometer (Seca, Germany). Participants were instructed to remove all footwear and stand underneath the stadiometer, feet together, heels down and facing away from the wall. Participants were requested to stand up straight and place their heels, buttocks and scapulae in contact with the wall. Participants were asked to relax their shoulders with their arms and hands hanging loosely by their sides. The head of each participant was angled so that it conformed to the Frankfort Plane (so to horizontally align the ear opening to the lower border of the eye socket) (as advised by BASES, 1997:46). The headboard was lowered until it made contact with vertex of the skull. Participants were asked to stand-up straight and inhale deeply as the researcher applied gentle pressure beneath the mastoid to stretch the participant upwards to their tallest stature (BASES, 1997).
2.4.2 Mass Measurement

Using a calibrated beam balance scale (Seca, Germany), body mass was then measured to the nearest 0.1 kg. Participants were weighed bare foot and wearing minimal clothing.

2.4.3 Auscultation – Brachial Pressure Measurement (Blood Pressure)

Participants were seated, and had been rested for five to ten minutes prior to the measurement. The instruments required were a sphygmomanometer consisting of a blood pressure cuff, a hand held continuous wave Doppler (Huntleigh Healthcare D900, UK) and acoustic gel. The cuff was placed around the participant’s upper right arm, acoustic gel was applied over the brachial artery; the earpieces of the stethoscope were placed in line with the researcher’s auditory canals and then the Doppler was placed over the brachial artery until a metallic tapping sound was apparent. The cuff was then inflated to about 20 to 30 mmHg above the estimated systolic pressure until a sharp thud was heard, this was caused by a sudden rush of blood released as the artery opens and provided the systolic pressure measurement (as instructed by Heyward, 2006). The cuff then needed to be gradually deflated until the tapping sound disappeared, at this moment the value was recorded as it signalled the diastolic pressure measurement.

2.4.4 Gas Calibration and Analysis

Oxygen uptake and RER were measured using an online breath by breath gas analyser (Oxycon Pro, Germany). Before each testing session the Oxycon computer settings were updated with current room temperature (°C) and humidity (%) measurements; following this, the gas analyser was calibrated using a calibration cylinder with known gas concentrations of O₂ 19.4%, CO₂ 0.6% and Nitrogen Balance. Patient details were then entered into the computer database (e.g. name,
identification code, height, body mass, age and gender), this would allow the gas analyzer to take into account the patients physiological variables when producing the results. Once the participant had boarded the treadmill they were fitted with a heart rate monitor (Polar Electro, Kempele, Finland) and a suitably sized Hans Rudolph face mask. As soon as the warm-up was initiated gas analysis was activated and then re-started at the beginning of the actual test. Breath by breath readings of $O_2$ uptake and RER were taken, following termination of each test all data was exported to an excel file ready for analysis. $O_2$ uptake ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was averaged over the last 30 seconds of each three minute RPE stage ready for analysis.

2.5 Procedures

2.6.1 Test Procedures

Each participant performed four laboratory-based exercise tests over a period of ten days to allow for a recovery period of approximately 48hrs between exercise bouts. All testing was performed on a motorised Woodway PPS55 Sport-I treadmill (USA). Height and body mass data were recorded at before each exercise test. The ambient temperature in the laboratory was fluctuated between 18-25°C, over the course of the study and air-conditioning was activated for added comfort.

Preceding all four test protocols a three minute warm-up phase was implemented at 3.13 m/s (7 mph) at 0% gradient. On-line respiratory gas analysis provided readings every five seconds throughout each exercise test via an automatic breath-by-breath gas calibrator system (Oxycon Pro, Germany). Prior to every test this system was calibrated against known concentrations of cylinder gases and a 3-1 syringe (for flow volume) in line with the manufacturer’s guidelines. The Expired air was collected continuously using a Hans Rudolph face mask. A wireless chest strap telemetry system (Polar Electro, Kempele, Finland) continuously recorded HR during each exercise trial. All screens and instruments displaying speed, gradient and physiological output were concealed from the participants during each exercise test.
so to prevent any external influences causing participants to recreate those settings in the future tests (Eston et al., 2008).

Prior to the initial exercise test, participants were familiarised with the treadmill and equipment, and introduced to the Borg 6-20 scale. Overall exertion (Undifferentiated RPE) was continuously reported during each of the exercise tests. Before each exercise session, participants were read, and asked to read standardised instructions of how to use the Borg scale and how to apply it within the test procedure, further verbal clarification was given if necessary.

The instructions conveyed to the participants detailing how to gauge the RPE scale provided verbal anchors which associate relevant sensations to be expected at a particular RPE; for example RPE 9 was explained as “a very light effort; for a normal healthy person it is like walking or cycling at a comfortable pace for quite a while” as provided by Borg (1998) (Appendix D). The use of such descriptors and associations are employed to stimulate memory anchors to past exercise experiences and thus draw parallels to similar sensations felt to those requested on the Borg 6-20 RPE scale (Gearhart, 2008). The RPE scale was positioned within sight and reach of the participant throughout each exercise test; the participant could verbally report RPE or point to the selected RPE.

2.6.2 Graded Treadmill protocols

Perceptually Regulated Graded Exercise Tests (PGXTs)

The first three (PGXT) tests were based on RPE sub-maximal production protocols consisting of four self-regulated exercise intensities 9, 11, 13, and 15 according to the Borg 6-20 RPE scale (Borg, 1998), prescribed in an incremental and continuous fashion. Participants were required to self-regulate their exercise intensity via the
treadmill’s built-in speed dials, in accordance with 4 prescribed RPE levels (9, 11, 13, and 15).

To successfully commensurate with standard sub-maximal graded exercise test protocols for healthy individuals, PGXTs required a step change in exercise intensity and metabolic cost of 1 – 2 METS (3.5–7.0 ml·kg⁻¹·min⁻¹) at each RPE stage following participant speed regulation (ACSM, 2006; Eston et al., 2008). This was achieved through gradient regulation conducted by the researcher on instruction by the participant. Participants instructed the researcher to reduce or increase speed to attain the prescribed intensity; this was done as accurately as possible around the following, recommended gradient adjustments (see table 2.1 and figure 2.1 overleaf).

Table 2.1.  
*Speed to Gradient ratios which elicited the recommended step changes in oxygen cost (MET) at each self-regulated RPE range.*  
*Gradient regulated in unison with speed.*

<table>
<thead>
<tr>
<th>Speed (km/h)</th>
<th>Gradient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>2.6</td>
<td>1.0</td>
</tr>
<tr>
<td>3.9</td>
<td>1.5</td>
</tr>
<tr>
<td>4.2</td>
<td>2.0</td>
</tr>
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<td>5.5</td>
<td>2.5</td>
</tr>
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<td>3.0</td>
</tr>
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<td>3.5</td>
</tr>
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<td>4.0</td>
</tr>
<tr>
<td>10.7</td>
<td>4.5</td>
</tr>
<tr>
<td>12.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Participants were initially instructed to exercise to an intensity that they perceived corresponded to an effort equivalent of nine on the RPE scale. Participants were then given 3 min to habituate to that exercise level, adjusting the speed when necessary. Fletcher et al. (2001) advocate that a period of ≥2 min at a constant effort level is required in order for \( \dot{V}O_2 \) levels to reach a steady state, and thus allowing for accurate recordings. At the end of three minutes the participant was instructed to progress to a level of effort they perceived to equate to RPE 11. This process was repeated for RPEs 13 and 15, respectively.

Graded Exercise Test (GXT)

The fourth test was a GXT designed to establish \( \dot{V}O_2_{\text{max}} \) by achieving maximal exertion. The Bruce protocol was selected, a continuous and incremental protocol which places heavy emphasis on gradient. The Bruce protocol ascends in both speed and gradient at 3 min stages; initialising exercise intensity at a speed of 1.7 mph and a gradient of 10%, intensity incrementally increases in gradient by 2% every 3 min in-
line with simultaneous increments in speed of 2.5, 3.4, 4.2, 5.0 and 5.5 mph. It should be noted that the attainment of $V_{O2\text{max}}$ is highly individualised and the participant can claim maximal exertion at any time or stage of the test.

The Bruce protocol is notorious for quickly achieving $V_{O2}$, however in healthy participants it is expected to sustain a GXT for 9-10 min which conforms to the recommended guidelines for optimal test duration (Myers & Bellin, 2000; ACSM, 2006). The second reason this protocol was selected is that it statistics suggest that it is the most popular exercise test adopted within a clinical field, thus yielding a great deal of functional and prognostic data generating significant normative values (Myers & Bellin, 2000). However, Myers and Bellin (2000) draw attention to scepticism surrounding the Bruce protocol within clinical trials as it contains large and unequal increments of exercise which serve to disrupt the standard linear relationship held between $V_{O2}$ and work rate, thus causing an over-prediction of metabolic equivalents. Yet, Myers and Bellin (2000) go on to add that such a trend is more applicable to cardiac patients, particularly those with cardiovascular disease, rather than healthy individuals. Such allegations stirred Green et al. (2002) in to investigating physiological responses to both incline and level exercise in participants producing RPEs associated with 50% and 70% $V_{O2\text{max}}$. RPEs were first examined in a GXT (Bruce test protocol) to volitional exhaustion, participants were then required to produce RPEs corresponding to 50% and 70% $V_{O2\text{max}}$ as estimated from the maximal GXT; no significant differences were found in HR and $V_{O2}$ from the high incline production mode to the values estimated from the GXT, however significant differences ($p \leq 0.05$) were found between estimated values and level treadmill values Green et al. (2002). It was therefore concluded that estimation-production trials correspond better when incline was similar to that used in the Bruce protocol (Green et al., 2002).

During the exercise bout, the participant was instructed to report their RPE during the final 30 seconds of each 3 min stage of the GXT. Conclusion of the test (maximal effort/$V_{O2\text{max}}$) was determined when attainment of the physiological measurements
and RPE matched the criteria highlighted by BASES (1997:64): a levelling off of \( \dot{V}O_2 \) consumption with increasing exercise intensity; a respiratory exchange ratio (RER) of \( \geq 1.15 \); \( \geq 90\% \) of maximum heart rate (HR\(_{\text{MAX}}\): 220 - age); or a HR value within ±10 bpm of the age-predicted maximum; volitional exhaustion; and an RPE of 19-20.

### 2.7 Statistical Analyses

The mean \( \dot{V}O_2 \) values (dependent variable) recorded over the final 30 seconds of each RPE level across all three PGXT production trials provided the basis for analysis. The mean \( \dot{V}O_2 \) values recorded from the last 30 seconds of each RPE level for each individual, across all three trials were subjected to multiple regression analysis. Linear regression analysis was performed using the equation \( \dot{V}O_2 = a + b \ (\text{RPE}) \) to predict each participants estimated \( \dot{V}O_{2\text{max}} \) from their sub-maximal exercise bouts, this procedure was carried out for all 54 of the sub-maximal production trials. The \( \dot{V}O_2 \) values from perceptual RPE ranges of 9-15, 9-11 and 9-13 were extrapolated to RPE\(_{19}\) and RPE\(_{20}\) to predict \( \dot{V}O_{2\text{max}} \). The RPE ranges 9-15, 9-11 and 9-13 were all regressed to see if \( \dot{V}O_{2\text{max}} \) predictions could be made with repeatable accuracy from the lower perceptual ranges, if this was proved to be the case then patients could be exercise tested at less strenuous intensities to predict maximal aerobic capacity. Coefficients were extrapolated to both RPE\(_{19}\) and RPE\(_{20}\) to assess which RPE predictive measure was the most consistently accurate with actual \( \dot{V}O_{2\text{max}} \). Although theoretically RPE\(_{20}\) indicates maximal exertion, St Clair Gibson et al. (1999) and Faulkner et al. (2007) state that RPE\(_{19}\) is most commonly reported upon a participant reaching volitional exhaustion.
Of note, RPE_{19} was the average peak value reported across GXTs within this study.

The Shapiro-Wilk statistic and relevant descriptive statistics were employed to assess for normal distribution of the dependent variable (\( \dot{V}O_2 \) value) across the sample. The three predicted \( \dot{V}O_{2\max} \) values for each participant were then compared to the actual \( \dot{V}O_{2\max} \) values obtained from the maximal GXT using a one-way repeated measures ANOVA. Post-hoc analyses were performed via paired t-tests by implementing a Wilcoxon signed ranks test/s in the event of any significant effects, concurrently the Bonferroni adjustment was employed to protect against an increased risk of Type 1 error which is common when performing multiple comparisons. The 95% LoA between the predicted and actual \( \dot{V}O_2 \) values were also quantified in order to assess the validity of the perceptually-derived \( \dot{V}O_{2\max} \) predictions across the three repeated production trials, and also whether LoA improved with practice.

Due to the limitations of using correlation coefficients as the principle means of reliability testing, as highlighted by Bland and Altman (1986) and Nevill and Atkinson (1997), it is advocated that the LoA technique be used instead of, or alongside the more conventional measures of assessing agreement such as correlation coefficients \( (r) \). It is expressed that \( r \) values solely measure the strength of a relation between two variables in ignorance of the agreement between them Bland and Altman (1986). It is stated that in order to validate findings, both perfect agreement and perfect correlation provide the most powerful statistics; however \( r \) values solely base reliability on the relative position of scores across the number of trials performed,
whereas the 95% LoA considers trial-to-trial variability, arguably satisfying reliability more rigorously (Bland & Altman, 1986; Lamb et al., 1996).

Therefore, to assess the reproducibility of $\dot{V}O_2$ at a given RPE, Bland and Altman’s (1986) 95% Limits of Agreement (LoA) technique was used to accommodate for levels of bias and random error by quantifying oxygen consumption at each prescribed RPE across all three production trials.

As advocated by Nevill and Atkinson (1997), the more traditional intra-class correlation coefficients (ICC) were calculated using a two-way mixed effects model in order to quantify the consistency of the $\dot{V}O_{2\text{max}}$ predictions between consecutive trials (trial 1 – trial 2 and trial 2 – trial 3), thus assessing levels of reliability. Furthermore, Pearson’s correlation coefficients were observed to confirm heteroscedasticity (assessing whether the test-retest differences were unrelated to the mean of the two trials), and so a significant correlation ($p \leq 0.05$) was necessary (Nevill and Atkinson, 1997).

All statistical analyses were performed using SPSS for Windows (version 14.0) and the alpha values were set to 0.05.