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The Measurement of Oxygen Uptake Kinetics in Children

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Abstract

Traditional approaches to exercise testing in children may not provide the most appropriate measures of a child's physiological responses to exercise, partly because they do not reflect children's normal intermittent activity patterns. The measurement of the rate and magnitude of change of oxygen uptake to dynamic exercise, oxygen uptake kinetics ($\dot{V}O_{2\ KINETICS}$), provides an alternative approach to exercise testing. A submaximal, intermittent, pseudo-random binary sequence (PRBS) exercise test to measure $\dot{V}O_{2\ KINETICS}$, may provide a useful method of measuring the metabolic responses of children to exercise.

Traditional methods used in the analysis of $\dot{V}O_{2\ KINETICS}$ require the fitting of explicit models in order to characterise the data. These models have not however been validated for use in children. As the responses to the PRBS protocol are analysed in the frequency domain, explicit models and their physiological correlates are not required to characterise the data. Another potential problem in the measurement of $\dot{V}O_{2\ KINETICS}$ in children are the small work rate changes that can be employed to stimulate the exercise response whilst constraining the test to the aerobic range. In respiratory gas measurement, breath-by-breath variability (noise) can be large in comparison to the magnitude of the metabolic response and this signal noise can obscure some characteristics of the response.

The aim of the study was to develop appropriate measurement techniques to reduce the effects of breath-by-breath variability and to apply the techniques to the measurement of $\dot{V}O_{2\ KINETICS}$ in children. The main experimental study compared the $\dot{V}O_{2\ KINETICS}$ of children with those of adults.

Ten children (3 females) in the age range 8 to 13 and twenty adults (10 females) in the age range 20 to 28 years completed a PRBS test to measure $\dot{V}O_{2\ KINETICS}$ and an incremental ramp protocol on a cycle ergometer (Bosch 550 ERG) to establish $\dot{V}O_{2\ MAX}$, T_{VENT} and delta efficiency. Breath-by-breath respiratory gas analysis was undertaken using a respiratory mass spectrometer (MGA1100). Estimates of alveolar gas exchange were made using the algorithm of Beaver et al. (1981) and a *post hoc* value of an effective lung volume was calculated to minimise the breath-by-breath variability. A cross-correlation technique (CC) was used to filter out the effects of anomalous (non-physiologic) $\dot{V}O_2$ responses recorded during the PRBS protocol. Subsequent Fourier analysis of the auto-correlation and CC functions provided a description of $\dot{V}O_{2\ KINETICS}$ in the frequency domain in terms of amplitude ratio and phase delay over the frequency range of 2.2 – 8.9 mHz.

At each of the frequencies assessed amplitude ratio was higher in children ($P<0.001$) than in either of the adult groups. Phase delay was also significantly shorter in children compared to adults males ($P<0.01$) and adult females ($P<0.001$) but this effect was not identifiable at any specific frequency. Maximal oxygen uptake was not significantly different in adult males (42.5 $ml \cdot kg^{-1} \cdot min^{-1}$) and children (44.7 $ml \cdot kg^{-1} \cdot min^{-1}$) but was lower in adult females (36.9 $ml \cdot kg^{-1} \cdot min^{-1}$) than adult males ($P<0.01$) and children ($P<0.001$). Ventilatory threshold ($\%\dot{V}O_{2\ MAX}$) was not different between groups. Delta efficiency was significantly lower in children than adult males ($P<0.05$) and adult females ($P<0.01$).

These results support the contention that there are maturational differences between adults and children in the metabolic processes involved in the utilisation of oxygen during physical activity. It has been argued, theoretically, that in adults the control of $\dot{V}O_{2\ KINETICS}$ is driven by ATP demand in the skeletal muscle. As the mitochondrial capacity and the concentration of oxidative enzymes is higher in children than in adults it is likely that the controlling factor(s) for $\dot{V}O_{2\ KINETICS}$ in children also relates to some aspect of peripheral metabolism. It is suggested that the PRBS protocol, with appropriate noise reduction techniques, is considered a suitable method for investigating the metabolic responses of children to dynamic exercise.

Published work relevant to this thesis includes:

Journal Articles

(1998) Marven, S.S, Smith, C.M., Chapman, J.H., Claxton D.B., Davies, H.A., Primhak, R,A. and Powell, C.V.E. Pulmonary function, exercise performance and growth in survivors of congenital diaphragmatic hernia. *Archives of Diseases in Childhood* 78(2): 137-142.

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Conference Presentations/Abstracts

(1993) Claxton, D.B., Chapman, J.H. and Fysh, M. L. The alignment of ventilation and fractional gas concentration by two "breath by breath" gas analysis systems. British Association of Sport Science. *UK: Partners in Performance Didsbury*, Manchester.

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Abbreviations

ω	angular velocity
$\Delta FetN_2$	change in fractional end tidal N_2 concentration
$\Delta FetO_2$	change in fractional end tidal O_2 concentration
ΔVL	change in lung volume between breaths
$\Delta \dot{V}O_2$	change in rate of oxygen uptake
ΔWR	change in work rate
η	work efficiency
ACF	auto-correlation function
ACSM	American College of Sports Medicine
ANOVA	analysis of variance
AT	anaerobic threshold
ATP	adenosine triphosphate
ATS	American Thoracic Society
a-$\bar{v}O_2$ difference	arterio-venous oxygen difference
BASES	British Association of Sport and Exercise Sciences
βATP	beta adenosine tri-phosohate
C	creatine
CK	creatine kinase
CCF	cross-correlation function
CV	coefficient of variation
D	time delay
DC	direct current
DCR	dynamic calibration rig
e	the natural logarithm of numbers
EVL	effective lung volume
fb	frequency of breathing (respiratory rate) in breaths per minute
$FECO_2$	fractional concentration of CO_2 in total expired gas
FEN_2	fractional concentration of N_2 in total expired gas

FEO_2	fractional concentration of O_2 in total expired gas
$FetN_2$	fractional end tidal N_2 concentration
FetO2	fractional end tidal O_2 concentration
FEV_1	forced expiratory volume in one second
FIN_2	fractional concentration of N_2 in total inspired gas
FIO_2	fractional concentration of O_2 in total inspired gas
FRC	functional residual capacity
FVC	forced vital capacity
ICDH	isocitric dehydrogenase
[lactate]	lactate concentration
LBNP	lower body negative pressure
LBPP	lower body positive pressure
LDH	Lactate dehydrogenase
LT	lactate threshold
NLV	nominal lung volume
P	power
PO_2	partial pressure of oxygen
PD	phase delay
PC	phosphocreatine
$PETCO_2$	partial pressure end tidal CO_2
$PETO_2$	partial pressure end tidal O_2
PFK	phosphofructokinase
PHV	peak height velocity
Pi	inorganic phosphate
^{31}P-MRS	phosphorous nuclear magnetic resonance spectroscopy
^{31}P-NMR	phosphorous nuclear magnetic resonance imaging
PRBS	pseudo-random binary sequence
pMVV	predicted maximum voluntary ventilation
RCT	respiratory compensation threshold

RER	respiratory exchange ratio
RT-$\dot{V}O_2$	response-time of oxygen uptake determined at the onset of a ramp exercise protocol
S.D.	standard deviation
SDH	succinic dehydrogenase
SV	stroke volume
τ	the time constant (tau) of an exponential curve [$(1-e^{-1})x100$]
t	time
T	torque
TPR	total peripheral resistance
$t½\dot{V}O_2$	time to reach one half of the final oxygen uptake response
T_{vent}	ventilatory threshold
VCO_2	volume of CO_2 exhaled
$\dot{V}CO_2$	rate of CO_2 production
VE	volume of expired gas
$\dot{V}E$	rate of pulmonary ventilation
VEO_2	volume of O_2 expired
VEN_2	volume of N_2 expired
VI	volume of inspired gas
$VICO_2$	volume of CO_2 inspired
VIN_2	volume of N_2 inspired
VIO_2	volume of O_2 inspired
VN_2	nitrogen balance - difference between inspired and expired nitrogen volume
VO_2	volume of O_2 utilised
$\dot{V}O_2$	rate of oxygen consumption / oxygen uptake / oxygen utilisation
$\dot{V}O_{2(KINETICS)}$	oxygen uptake kinetics
$\dot{V}O_{2(max)}$	maximum rate of oxygen uptake or maximum aerobic power
$\dot{V}O_{2(peak)}$	highest rate of oxygen uptake achieved during an incremental test

$\dot{V}O_{2(SS)}$	steady state rate of oxygen uptake
$\dot{Q}$	rate of cardiac output

Preface

Paediatric exercise testing is an emerging area of exercise physiology, however secular changes in exercise performance throughout a child's growing years are not easily explained by conventional testing procedures. These procedures, which were originally designed for adults, generally rely on exhaustive tests which many people think are inappropriate for children as the results do not fully explain the large improvements in exercise performance over the growth period. In addition, the interpretation of the results is difficult because the effects of changes in body mass and structure cannot easily be distinguished from changes in physical fitness. There is therefore considerable potential for a sub-maximal exercise test which would provide information about the physiological systems responsible for oxidative metabolism in children but would not require scaling techniques to partition out the effects of growth.

The measurement of the rate of change in oxygen utilisation, oxygen uptake kinetics ($\dot{V}O_{2\ KINETICS}$), in response to changes in work rate provides a suitable technique for measuring the physiological response to sub-maximal exercise. If such a test were to be used in children the work rate perturbations would be small in magnitude with consequent small changes in oxygen utilisation. The characteristics of the response will therefore be difficult to discern from the respiratory noise inherent in the measurement of oxygen uptake using respiratory analysis apparatus. The first step in measuring $\dot{V}O_{2\ KINETICS}$ in children therefore would be to use techniques which minimise respiratory noise and enhance the signal to noise ratio.

A rationale for a study based on the measurement of $\dot{V}O_{2\ KINETICS}$ in children is provided in the two introductory chapters. The first chapter examines the developmental aspects of exercise performance. Chapter 2 provides an overview of $\dot{V}O_{2\ KINETICS}$ and Chapter 3 serves as a precursor to the materials and methods section (Chapter 4) as it addresses issues relating to the reliability of measuring $\dot{V}O_{2\ KINETICS}$. Chapter 4 describes in detail the protocols and procedures employed in Chapter 5. This concluding chapter is an experimental study which compares the $\dot{V}O_{2\ KINETICS}$ of children to those of young adults.

1. Developmental Exercise Physiology

The studies which look at the development of exercise performance in children are either longitudinal over the years of growth (Beunen and Malina, 1988), cross sectional comparisons of different age groups (Armstrong et al., 1991), or direct comparisons between adults with children (Armon et al., 1991). They all have problems inherent in comparing the responses of individuals of different size and development. This Chapter reviews the literature on developmental exercise physiology from the perspective of : issues relating to development, exercise performance, maximum oxygen uptake ($\dot{V}O_{2\,MAX}$), cellular energy metabolism, anaerobic threshold (AT), and work efficiency.

1.1. Growth, Maturation and Development

It has long been recognised that children are not simply small adults but have psychological and physiological characteristics distinct from those of the adult. The child is a continuously developing organism and the rate of development is governed not only by genetic instruction but also by environmental factors such as diet and physical activity patterns. The term development encompasses the concepts of both maturation and growth which are fundamentally different. Maturation implies progress toward the mature state, which varies with the biological system considered. Growth refers to changes in the size of the organism or of its parts. Both processes are probably under separate genetic regulation, yet they are related (Malina, 1988). One of the key difficulties when investigating the responses of children to exercise is in making appropriate comparisons between individuals of different size and maturational status. For example body size and composition change considerably during normal development, so that it is difficult to separate the effects of any intervention such as a training programme, from those associated with normal growth.

The practice of using chronological age, to classify the responses of groups of children to exercise, is the most accessible method of comparison, unfortunately this approach does not describe the subjects significantly well in terms of development. This is

particularly true during puberty when maturity related differences in strength, flexibility, speed, endurance, and power become more evident among children of the same chronological age (Maffuli, 1998). Chronological age is usually expressed as the age in years but for more general definitions the terms child, adolescent, youth, young adult or elderly are frequently used as age descriptors. The difficulty with these terms is that different researchers use slightly different conventions and care must be taken to note the age range of the groups particularly when making cross study comparisons. For most applications the use of chronological age alone to group subjects in terms of development is inappropriate, and researchers have adopted various methodologies in order to establish biological age by reference to the development of one or more of the following anatomical or physiological systems.

1.1.1. Stature

Children experience several periods of rapid growth as they develop and records of changes in stature and mass are the most fundamental of the measures used to asses biological age. The pubescent growth spurt, which lasts for about 2 years, is a particularly prominent growth characteristic. During this period a significant portion of adult size is attained and when the rate of change in stature is plotted against age, a distinctive peak occurs in boys at 13.5 to 14 years of age and in girls at 11.5 to 12 years of age (Cunningham, et al., 1984a). This peak is termed peak height velocity (PHV) and the age at PHV provides a useful reference point by which to classify children of similar biological age (Rutenfranz et al., 1982; Malina et al., 1997). The main problem in identifying the PHV is that it can only be ascertained with any certainty from accurate measurements of stature over a period of several years, and due to the large variability in the age and magnitude of the growth spurt among different individuals it imposes difficulties in studying the functional characteristics, such as the cardio-respiratory system, in relation to growth (Cunningham, et al., 1984a).

1.1.2. Sexual Maturation

The most frequently employed system for classifying biological age is the 5 point

secondary sex characteristic rating of puberty based on genital, breast and pubic hair development described by Tanner (1962). The assessment can be made directly by a trained observer, or reported after self assessment with reference to pictorial information. The onset of menarche in females can also provide a useful reference but the recall accuracy of its occurrence has been called into question in cross sectional studies (Pickles et al., 1998). Menarche is a rather late maturational event which occurs after PHV while most girls are in stage 4 of breast and pubic hair development (Marshal, 1978). The average difference between age at menarche and age at PHV is about 1.2 to 1.3 years (Tanner and Davies, 1985).

1.1.3. Skeletal Development

Radiographic rating of the left hand and wrist has been shown to provide reliable estimates of skeletal age (Tanner et al., 1975). Skeletal age is based on normal data derived from various sources and must be interpreted in relation to the original data source as there appears to be significant differences in skeletal development of children from different countries. Shephard et al. (1978), in a study of 770 children, concluded that skeletal age adds little to the description of physiological variables yielded by chronological age, stature, and mass. Coupled with the restriction that even minimal exposure to X rays are not normally justified, unless they are required on medical grounds, the value of skeletal age analysis in order to establish maturational status appears to be limited.

No single means of maturity assessment provides a complete description of maturity during adolescence (Armstrong and Welsman, 1994). However, it has been argued that although there is a variation in the development of secondary sex characteristics, skeletal maturity and peak height velocity within each sex during adolescence, these maturity indicators are sufficiently interrelated to indicate a general maturity factor during adolescence Malina (1988).

Classification of developmental status in terms of age, maturity and growth is only one fundamental issue in paediatric research which still requires to be resolved. The second

issue, which is key to many aspects of developmental biology, is that relating to scaling.

1.1.4. Scaling of Physiological Responses

Scaling refers to the statistical process of partitioning out differences in physiological responses which change in relation to size. Winter (1992) describes four arguments underpinning the need to consider the use of scaling (a) to compare individuals against standards for the purposes of assessment; (b) in comparisons between groups; (c) in longitudinal studies, especially with children, in which for example the effects of training have to be disentangled from the effects of development and; (d) in studies that explore the relationship between physiological variables and performance. There are four methods which are generally employed to partition out the effects of size or maturation, these are ratio standards, regression standards, allometric analysis (power functions) and multilevel modelling (Winter, 1992; Armstrong and Welsman, 1994). The two most frequently employed methods are the ratio standard and allomretric analysis.

1.1.5. The Ratio Standard

An anthropometric variable such as body mass, stature or body surface area is used as a denominator for a physiologic response, typically $\dot{V}O_{2\ MAX}$, in an attempt to scale the response in relation to body dimensions. If an improper denominator is chosen, it is possible that the outcome will result in misleading results and erroneous conclusions (Rowland, 1998). For a simple ratio standard to be valid, the coefficient of variation for the body size measure divided by the coefficient of variation for the physiological measure must equal the Pearson product-moment correlation coefficient for the two variables (Tanner, 1949). When this is not the case a typical scenario (using body mass as the denominator) is that in a large group of subjects with varying body mass, one may observe a negative correlation between $\dot{V}O_{2\ MAX}$/kg and body mass. That is, smaller or lighter subjects will have a higher $\dot{V}O_{2\ MAX}$/kg than heavier ones (Rowland, 1998). In this case $\dot{V}O_{2\ MAX}$ and body mass do not increase proportionately. While larger individuals are penalised in the case of maximal capacity when a greater value

represents greater capacity, they are favoured in an examination of sub-maximal economy where lower values suggest better economy. These statistical artefacts and the errors that they introduce become even greater when comparisons between adults and children are made (Rogers et al., 1995).

1.1.6. Allometric Analyses

Growing children do not grow as geometrical entities, they change in shape and composition and in general become more linear, less bony and more muscular with age (Ross and Marfell-Jones, 1991). The development of children is said to be non-isometric, that is there are changes in the relative size of the segments as a proportion of overall size. The scaling technique that can be used to account for these type of changes is referred to as allometry. Allometric or power function equations describe curvilinear relationships between physiological variables (Welsman et al., 1996). With this approach any physiologic variable Y can be expressed relative to a body dimension (such as mass) X by the basic equation $Y = aX^b$, where b is the scaling exponent. In this case, if the physiologic variable Y changes in direct proportion to body mass, $b = 1.0$. If, as mass increases, Y does so at a slower rate, then $b < 1.0$, and if Y increases more rapidly than mass, $b > 1.0$. If Y declines as mass increases, the values for b are negative (Winter, 1992). The exponent b is derived from the slope of the regression on logarithmic (ln) transforms of data: $ln\ Y = ln\ a + b \cdot ln\ X$. Power function ratios can be constructed by dividing the physiological variable by the body dimension raised to the power b (Y/X^b). Power function ratios as they partition out body size differences correctly permit comparisons between groups in cross-sectional studies (Winter, 1992; Armstrong and Welsman, 1994).

Nevill et al., (1992) suggests that when modelling a physiological variable such as $\dot{V}O_{2\,MAX}$, stature as well as body mass must be incorporated into the allometric equation. This allows for the influence of body mass to be separated from the additional effect of the disproportionate increase in muscle mass. The effects of maturity also complicate the use of scaling techniques. In a study by Kemper et al. (1986) of early and

late maturing teenagers $\dot{V}O_{2\,MAX}$ relative to body mass was lower in early maturers than in late maturers. This is explained by a higher percentage of body fat in both males and females in the early maturing group as compared to the late maturers. Malina et al. (1997) also concludes that given the size differences between individuals of contrasting maturity status, it might be expected that scaling peak $\dot{V}O_2$ for body mass and stature would vary with maturity status of the child, and that there would be inter-individual variation in scaling coefficients during early and mid-adolescence.

The power functions calculated using allometric techniques in studies of children have consistently exceeded the theoretical predictions and several authors have concluded that there is no strong argument for abandoning the practice of expressing physiological variables in ratio with body mass (Bar-Or, 1983; Rowland, 1990). Nevill (1997) however, considers the ratio standard to be the best predictor of athletic performance in weight bearing activities, but when athletic performance is not weight bearing the body size denominator component of a scaled ratio is likely to be considerably less, if not absent and absolute $\dot{V}O_{2\,MAX}$ ($l \cdot min^{-1}$) is probably the best predictor.

Recent comment by Rowland (1998) considers that the issue of appropriate scaling is still not adequately resolved and suggests that experimental results are expressed as ratio standard and after application of allometric scaling techniques researchers should address any differences in conclusions created by the two techniques.

1.2. Exercise Performance

There are differences in exercise performance between children and adults. As children grow they become more able to perform physical work. For example, endurance running performance steadily improves (Massicotte et al., 1985; Cureton et al., 1997), and these faster times in distance events are linked to the process of development. In response to a standard sub-maximal exercise, heart rate, ventilatory equivalent for oxygen, mass-relative oxygen uptake, and ratio of respiratory rate to tidal volume, all decline as the child grows, features which are characteristic of the aerobic exercise training response (Rowland, 1995). Increases in absolute $\dot{V}O_{2\,MAX}$ essentially mirror the

increase in body size with high correlations between $\dot{V}O_{2\,MAX}$ and body mass or stature (Armstrong et al., 1991; Malina et al., 1997). Children as they grow also increase their ability to sustain high intensity exercise (Bar-Or, 1983).

It could be argued that these alterations in physiological responses might simply reflect increases in body size. There is however growing evidence that improvement in performance is not only related to size but is associated with both improved cardio-vascular function, and also maturity associated changes in the metabolic pathways essential for physical activity. In fact, there is only a poor relationship between endurance performance and mass relative $\dot{V}O_{2\,MAX}$ as children grow. For example, between the ages of 5 and 15 years, finish times in the 1-mile run improve approximately 100% in boys, yet $\dot{V}O_{2\,MAX}$ per kg body mass remain unchanged (Rowland, 1990). Tests of anaerobic power, for example the Wingate test, show improvements in anaerobic power which are faster than the increase in mass or stature suggesting that performance is at least independent of body dimensions (Rowland, 1995).

1.2.1. Maximum Oxygen Uptake in Children

As oxygen is a key component of cellular energy production, the measurement of oxygen utilisation ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) using respiratory gas analysis provides a convenient, indirect determination of energy expenditure. Maximum oxygen uptake ($\dot{V}O_{2\,MAX}$) is a measure of the highest rate that oxygen can be taken in and utilised by an exercising subject under standardised atmospheric conditions at sea level. The value can be expressed in *absolute* terms of $l \cdot min^{-1}$ or is scaled *relative* to an anthropometric measure as described earlier (Section 1.1.4). The $\dot{V}O_{2\,MAX}$ provides a quantitative statement of an individual's capacity for aerobic energy transfer and is an important determinant of an individual's ability to sustain high-intensity exercise for longer than 4 or 5 minutes (McArdle et al., 1991). Maximum oxygen uptake depends chiefly on venous O_2 content and circulation rate and can be expressed in terms of the Fick equation :-

$$\text{Maximal oxygen uptake} = \text{Maximal cardiac output} \times \text{Maximal arterio-venous oxygen difference} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where, cardiac output ($\dot{Q}$) is the total amount of blood pumped from the left ventricle each minute, and arterio-venous oxygen ($a\text{-}\bar{v}O_2$) difference is the average difference between the oxygen content of arterial and mixed venous blood.

There are several methods of obtaining estimates for $\dot{V}O_{2\,MAX}$, indirectly from running tests, or by prediction using sub-maximal exercise protocols, but the evidence suggests that there is no valid substitute for direct determination of $\dot{V}O_{2\,MAX}$ (Armstrong and Welsman, 1994). Maximum oxygen uptake is considered to have been attained in an incremental exercise test if there is a plateau in the oxygen response even though the exercise intensity continues to increase (Astrand and Rodahl, 1986). There has been significant debate over the efficacy of using this sole criteria as a index of establishing $\dot{V}O_{2\,MAX}$ in children as only a minority of children can sustain the high intensity exercise required during a maximal exertion test to establish a plateau in the $\dot{V}O_2$ response (Armstrong and Welsman, 1994). Most exercise physiologists, in the absence of a plateau, report the value as $\dot{V}O_{2\,PEAK}$, which is the highest oxygen uptake achieved during the incremental test. Several studies have however concluded that the highest $\dot{V}O_2$ of children who demonstrate a plateau response is indistinguishable from those that do not (Cooper et al. 1984; Rivera-Brown et al., 1992; Armstrong et al., 1996; Duncan et al., 1996). Several criteria are now considered as evidence to establish if $\dot{V}O_{2\,PEAK}$ can be accepted as a maximal indices. These criteria include: assessing visually if the child has exerted maximum effort (Armstrong et al., 1995), a maximum heart rate of approximately 195 beats·min^{-1} in cycle protocols or 200 beats·min^{-1} whilst running on a treadmill (Rowland, 1996), ventilation reaching 60% of maximum rate (Godfrey, 1974), or a respiratory exchange ratio (RER) greater than 1.0. The RER expresses the relationship of expired carbon dioxide to oxygen uptake, which, as the ratio increases above unity indicates the anaerobic contribution to the energy supply. Although high post exercise blood lactate levels of 6-7 mmol·l^{-1} may be considered as

an index of $\dot{V}O_{2\,MAX}$ having being achieved in children (Armstrong et al., 1996) this criterion has been challenged recently due to 1) relatively poor reliability, 2) the effects of different exercise protocols in stimulating lactate production to different degrees and, 3) possible differences in the motivation to exercise maximally (Pfitzinger and Freedson, 1997a; Tolfrey and Armstrong, 1995). In the absence of a plateau but where some of the criteria to accept $\dot{V}O_{2\,PEAK}$ as an index of maximal performance have been met, the term $\dot{V}O_{2\,MAX}$ may therefore be considered appropriate.

The research investigating the secular trend in $\dot{V}O_{2\,MAX}$ ($l \cdot min^{-1}$) has been reviewed by Bar-Or (1983) and Armstrong and Welsman (1994). Armstrong and Welsman's review represents 10,154 $\dot{V}O_{2\,PEAK}$ data points from subjects aged 8-16 years on either cycle ergometer or treadmill. The trend in the data shows a gradual increase in boys' $\dot{V}O_{2\,PEAK}$ in relation to chronological age. In girls there is a similar but less consistent trend. From age 8 to 13 years, girls' $\dot{V}O_{2\,PEAK}$ appears to increase with chronological age. At age 13 to 15 years, several of the cross-sectional studies reviewed, suggest a levelling-off or a fall in $\dot{V}O_{2\,PEAK}$. Further analysis of the data (Armstrong and Welsman, 1994) indicates that the treadmill determined $\dot{V}O_{2\,PEAK}$ values of 10 year old males are 12% greater than those of similarly aged females. This difference rises to 37% at 16 years of age. A similar trend is shown for cycle ergometer determined $\dot{V}O_{2\,PEAK}$ values. The boys' aerobic power is 2% higher than females at age 10 years rising to 37% higher at age 16 years. Similar conclusions were also drawn in the review by Bar-Or (1983) who considered the relationship between $\dot{V}O_{2\,MAX}$ and chronological age in 3,910 girls and boys of age range 6 to 18 years old. As the child grows there is a concomitant increase in $\dot{V}O_{2\,MAX}$. Until age 12 years, values increase at the same rate in both sexes, although boys have higher values from age 5 years. Maximum oxygen uptake levels off in females above age 14, but continues to rise until the age of about 18 in boys.

The relationship between increases in $\dot{V}O_{2\,MAX}$ and maturity may be explained by the findings of Armstrong et al. (1991) as probably due to greater muscle mass (indicated in his study by a higher body mass but no difference in skin fold thickness) in more mature

boys and higher haemoglobin concentration. Armstrong et al. (1991) concludes that boys' mass-related $\dot{V}O_{2\,PEAK}$ was independent of sexual maturity stage. In girls, the trend was reported to be similar with mass-related $\dot{V}O_{2\,PEAK}$ generally being independent of maturity stage when established from both treadmill and cycle ergometer administered maximum exercise protocols. The results of Armstrong et al.'s (1991) study shows the average values for $\dot{V}O_{2\,PEAK}$, for 113 boys of age range 11 to 16 years, of 2.31 $l \cdot min^{-1}$ and for 107 girls of the same age of 1.90 $l \cdot min^{-1}$ recorded in response to a maximal treadmill exercise test. The $\dot{V}O_{2\,PEAK}$ attained for the same groups undertaking maximal cycle ergometer tests were 2.05 and 1.72 $l \cdot min^{-1}$ for boys and girls respectively.

The results of a study by Welsman et al., (1996) show that, $\dot{V}O_{2\,PEAK}$ relative to body size remains constant throughout development in boys, while there is a tendency for relative $\dot{V}O_{2\,PEAK}$ to decline with growth in girls. The results from linear and log-linear adjustment models shows that $\dot{V}O_{2\,PEAK}$ increases from pre-puberty to adulthood in males. In females, $\dot{V}O_{2\,PEAK}$ appears to improve between pre-puberty and circum-puberty with levels then remaining constant into adulthood rather than decreasing. Considering the improvement in endurance performance over this age range, $\dot{V}O_{2\,MAX}$ per kg should not be expected to be predictive of endurance performance in any group of children of different biological age (Rowland, 1990).

The data recorded by Armstrong et al. (1991) using a maximal treadmill protocol shows the average $\dot{V}O_{2\,PEAK}$ values for 113 males of age range 11 to 16 years of 49 $ml \cdot kg^{-1} \cdot min^{-1}$, and 41 $ml \cdot kg^{-1} \cdot min^{-1}$ for 107 females of the same age range. The results recorded on the same groups using a maximal cycle ergometer protocol were 43 $ml \cdot kg^{-1} \cdot min^{-1}$ and 37 $ml \cdot kg^{-1} \cdot min^{-1}$ for boys and girls respectively.

1.2.1.1. Factors Determining Maximum Oxygen Uptake in Growing Children

Maximum heart rate is approximately 200 $beats \cdot min^{-1}$ in children and begins to decline from this maximal level at the age of approximately 20 years at a rate approximately equal to one beat per year. Since maximum heart rate decreases with age (Robinson,

1938) any centrally mediated improvement in $\dot{V}O_{2\,MAX}$ must be a function of stroke volume (SV) (with reference to the Fick equation Section 1.2.1 Equation 1). In a longitudinal study of the cardio-respiratory function in circum-pubertal boys Cunningham et al. (1984b) used multiple regression analysis to examine whether the increase in $\dot{V}O_{2\,MAX}$ associated with development, could be accounted for by changes in maximum SV or by changes in maximum a-$\bar{v}O_2$ difference. The findings indicated that $\dot{V}O_{2\,MAX}$ was determined primarily by the size of the SV between the ages of 10 and 15 years. It was also observed that an increase maximal a-$\bar{v}O_2$ difference was a critical component of the increased $\dot{V}O_{2\,MAX}$ in the year preceding PHV. This suggests that maturation-dependent changes of oxidative enzymes and capillary to fibre ratio may be an important component of the increase in $\dot{V}O_{2\,MAX}$ ($l \cdot min^{-1}$) as well as the increase in SV associated with increased body size. It appears that the increase in muscle mass during development occurs first, followed by an increase in SV to meet the new metabolic capacity.

Maximal a-$\bar{v}O_2$ difference is limited by the haemoglobin content of the blood. Haemoglobin concentration is significantly correlated with age (Armstrong et al., 1991) in male and female children of age range ~ 11-16 years, and significantly correlated with $\dot{V}O_{2\,PEAK}$ ($l \cdot min^{-1}$) in both boys and girls. In relation to maturity, girls show no differences at the different stages of development, but the boys of Tanner stage 5 had significantly higher blood haemoglobin concentration (15.1 $g \cdot dl^{-1}$) than the boys of Tanner stages 3, 2, 1 and all the girls (range 11.8 to 14.0 $g \cdot dl^{-1}$), (Armstrong et al., 1991).

Despite their lower blood haemoglobin concentration the maximal a-$\bar{v}O_2$ difference of children is comparable to that of adults (Eriksson, 1972) because children are able to extract almost all of the oxygen circulating through the working muscles.

1.2.2. Cellular Energy Metabolism

The maximal concentration of blood lactate is positively related to age and seldom exceeds 6-8 $mmol \cdot l^{-1}$ in the first decade of life (Robinson, 1938; Astrand, 1952; Wirth et

al., 1978; Matejkova et al., 1980) as compared to 11 to 13 $mmol \cdot l^{-1}$ in adults (Astrand, 1952). There is considerable inter-individual variability in this measure and Cumming et al., (1980) recorded maximum serum lactates in young children of up to 14 $mmol \cdot l^{-1}$. It has been suggested by Pfitzinger and Freedson (1997a) that protocols are required to optimally stimulate the subject's glycolytic capacity in order to establish trends in maximum blood lactate between children and adults.

In sub-maximal exercise, children show lower lactate concentrations in blood and muscle during exercise at the same relative intensity when compared to adults (Macek et al., 1976; Mahon et al., 1997). In an assessment of pre-pubertal, pubertal and post-pubertal males and females Wirth et al., (1978) have demonstrated that sub-maximal exercise-induced increments in lactate are dependent on maturity. Blood lactate at a fixed percentage of $\dot{V}O_{2\,MAX}$ however do not account for differences among individuals with respect to the onset of the lactate threshold (LT).

The findings that children largely demonstrate lower peak muscle and blood lactate levels during exercise have been interpreted to indicate that children have less ability to rely on non-oxidative energy sources. The reliance of children on aerobic metabolism, rather than non-oxidative energy sources, can be attributed to enhanced oxidative metabolism and/or poor anaerobic metabolism. The evidence which examines this proposition is detailed in studies of cellular energy metabolism using either phosphorous nuclear magnetic resonance spectroscopy (^{31}P-MRS) or muscle biopsy techniques.

Phosphorous nuclear magnetic resonance spectroscopy provides a safe, non-invasive means of monitoring intracellular inorganic phosphate (Pi), phosphocreatine (PC), β adenosine tri-phosohate (βATP) and pH, and has been used in studies of children (Taylor et al., 1997; Zanconato et al., 1993). These studies have shown that children have both an increased oxidative capacity (Taylor et al., 1997) and a decreased anaerobic capacity (Zanconato et al., 1993), when compared with adults. These difference are only evident in the higher intensity range of exercise. In the investigation of Zanconato et al. (1993), 10 children (of age range 7-10 years) were compared to 8

adults (of age range 20-42 years). The Pi/PC ratio was reported to be 0.54 (± 0.12) during maximal exercise in children compared to 2.00 (± 0.79) in adults. The 27% lower Pi/PC ratio in children suggests that children are less able than adults to rephosphorylate ATP by anaerobic metabolic pathways during high intensity exercise.

The evidence from muscle biopsy analysis of cellular metabolism supports the findings of the ^{31}P-MRS studies. There is evidence of reduced phosphofructokinase (PFK) activity in boys compared with untrained, trained and athletic adults (Eriksson, 1972). Berg et al. (1986) compared skeletal muscle enzyme profiles in children with age and sexual maturation: a pre-pubertal group (6.4 years ± 2.1); a circum-pubertal group (13.5 years ± 1.3 years) and a post pubertal 17.1 ± 0.8 years). Significant increases were found in the glycolytic enzymes aldolase and pyruvate kinase, between pre- and post-pubertal groups. Lactate dehydrogenase (LDH) activity was also higher in the circum- and post pubertal groups than in the pre-pubertal group. It was highest in circum-pubertal group. These results were not confirmed by Haralambie (1982) who did not establish any differences in glycolytic enzyme activity between 14 adults of age 22 to 42 years, when compared with 14 children aged 13 – 15 years. The explanation suggested for this finding was that the adolescents used in the study of Haralambie (1982) were older than those in the study of Eriksson (1972) this explanation would not now seem appropriate in light of the evidence of Berg et al. (1986).

In comparison to adults, children have been shown to possess elevated levels of the oxidative enzymes succinic dehydrogenase (SDH) (Eriksson, 1972; Eriksson, 1980), fumarase (Haralambie, 1982; Berg et al., 1986) and isocitric dehydrogenase (ICDH) (Haralambie, 1982). In the study of Erikson (1972) 31 boys, in the age range ~11 to ~16 years had average SDH activity levels of 5.74 $\mu moles \cdot g^{-1} \cdot min^{-1}$ which compared favourably with a group of 12 trained men of age range ~17 to ~30 years (6.00 $\mu moles \cdot g^{-1} \cdot min^{-1}$). The average SDH activity level for 12 untrained men aged 24 – 30 was only 3.60 $\mu moles \cdot g^{-1} \cdot min^{-1}$.

Therefore the lower (~ 50%) PFK activity found in children (Eriksson, 1972) suggests limitations in anaerobic metabolism while improved oxidative enzyme activity suggests

a greater reliance on aerobic metabolism in children.

The reliance, in children, on aerobic metabolism rather than non-oxidative energy sources, can therefore be attributed to both enhanced oxidative metabolism and as a consequence of poor anaerobic metabolism. This is reflected in the different PFK to ICDH ratios of 1.633 in adults and 0.844 in children observed by Haralambie (1982).

Evidence from measurement of muscle fibre types in children and adults confirms the conclusions from the biochemical data and ^{31}P-MRS data. Boys have a higher proportion of slow twitch fibres compared to untrained men, and interestingly have fibre type profiles similar to aerobically trained adult males (Eriksson, 1972). More recent evidence also supports an age-dependent increase in glycolytic Type 2b fibres over the life span (Kriketos et al., 1997). Bell et al. (1980) measured fibre type composition in 13 six year olds using muscle biopsy techniques, and concluded that the fibre type distribution pattern and ultra-structure of skeletal muscle in six-year-olds was not different from either older children or adults involved in endurance training programmes. Based on ultra-structural parameters, relative volume densities of mitochondria and intra-cellular lipid, the children demonstrate equivalent or slightly greater capacity for oxidative metabolism than do adults. The training effects on skeletal muscle fibre area and distribution and on skeletal muscle enzyme activity has also been shown to be similar in adolescent boys (Fournier et al., 1982) when compared to adults (Golnick et al., 1973).

Although the metabolic pathways responsible for high intensity exercise have been shown to be different for children and adults, the effect of these peripheral factors on the exercise response to sub-maximal exercise is less well documented.

1.2.3. Anaerobic Threshold

During progressively increasing exercise, a point is reached at which exercise metabolism is no longer sustained by aerobic metabolism alone. The increasing requirement for ATP is supplemented by an increased rate of glycolysis with a concomitant increase in lactate production. The metabolic rate ($\dot{V}O_2$) at which lactate

production exceeds its removal from the blood is referred to as the lactate threshold (LT). It is considered that the rapid efflux of CO_2 from the cell generated by the buffering of the increasing [lactate] by bicarbonate will be evident quickly in lung gas exchange (Wasserman et al., 1987). This, together with the increased respiratory drive, which initially increases proportionately with CO_2, is detectable using respiratory gas analysis, and some physiologists use the $\dot{V}O_2$ at which pulmonary ventilation initially accelerates out of proportion to the metabolic load, rather than the point of increase in blood lactate, as an index of the anaerobic threshold. The analysis of the ventilatory response to detect the AT is referred to as the ventilatory threshold (T_{vent}).

1.2.3.1. Lactate Threshold

Pfitzinger and Freedson (1997b) suggest caution when making inter-study comparisons of LT as the measurement is specific to the exercise protocol used, the criteria adopted to determine it and the method used to assay the sample.

In spite of the fact that children produce less lactic acid at all work intensities (both sub-maximal and maximal) the LT of the two groups, determined from muscle lactate measurements are remarkably similar (Eriksson et al., 1971). Lactate thresholds (the point of rapid increase in blood lactate concentration) have been measured of 78.6 ± 3.4 and 63.7 ± 1.7 $\%\dot{V}O_{2\,MAX}$ in highly trained and untrained 11 - 12 year old boys respectively (Atomi et al., 1986). Rostein et al. (1986) assessed the effects of training on the AT in a group of twenty eight 10.2 – 11.6 year old boys and recorded pre-training LTs of 82.2 ± 7.0 and 79.9 ± 3.8 $\%\dot{V}O_{2\,MAX}$ in a training and a control group respectively, and a post training LT of 77.9 ± 5.5 $\%\dot{V}O_{2\,MAX}$ in the 16 boys who undertook 9 weeks of training. The $\dot{V}O_{2\,MAX}$ of the trained group increased by 8%. It is likely that this group of boys were aerobically fit as their pre-training $\dot{V}O_{2\,MAX}$ was 55.6 $ml \cdot kg^{-1} \cdot min^{-1}$. The LT ($\%\dot{V}O_{2\,MAX}$) results recorded by Rostein et al. (1986) were reported to be similar to adult elite endurance runners performing the same protocol. This compares with adult values of 69.9% $\dot{V}O_{2\,MAX}$ in young adults (Farell et al., 1979) and 43.6-49.3 $\%\dot{V}O_{2\,MAX}$ in middle aged men (Davis et al., 1979).

1.2.3.2. Exercise at a Predetermined Lactate Level

Exercise at a predetermined [lactate] has been reported for children between the ages of 12 and 14 (Williams and Armstrong, 1991). If an absolute value is used to represent LT in children, the 2.0 or 2.5 $mmol.l^{-1}$ concentration would be more appropriate than the 4.0 $mmol.l^{-1}$ level which has been used as an indicator of endurance performance in adults (Foxdall et al., 1996). However, any fixed concentration may be inappropriate because it appears likely that LT changes in the individual child over time, so any comparison of children at different stages of maturity using a fixed lactate concentration could be misleading (Pfitzinger and Freedson, 1997b).

1.2.3.3. Ventilatory Threshold

Given the ethical considerations of collecting blood lactate in children, the use of a noninvasive method to estimate LT in children is attractive.

Normal values for T_{vent} have been reported in children to be as low as 47.8 (± 11.7) $\%\dot{V}O_{2max}$ (McConnell et al., 1992) most studies using larger numbers of children report values in the range 58 – 75 $\%\dot{V}O_{2\,MAX}$ (Washington, 1993).

In a study where direct comparisons have made between boys and men, no difference was identified in T_{vent} when it is expressed as a percentage of $\dot{V}O_{2\,MAX}$ (Mahon et al., 1997). Nine boys of average age 10.5 (± 0.7) years and nine men of age 25.3 (± 2.0) years performed incremental exercise on a cycle ergometer. The results are shown in Table 1.1.

Table 1.1. Cardiorespiratory responses at T_{vent}. Mean (standard deviation (S.D.)). From Mahon et al. (1997).

Variable	Boys (n=9)	Men (n=9)
$\dot{V}O_2$ ($l \cdot min^{-1}$)	1.17 (1.06)	2.67 (0.47)*
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	32.0 (3.7)	33.8 (4.1)
$\%\ \dot{V}O_{2\,MAX}$	67.2 (3.5)	67.3 (4.9)

* Significant difference between groups.

Similar results were found in a previous study which compared young and adult female athletes specialising in different sports (Bunc and Heller, 1993). Ventilatory threshold

was determined in a treadmill exercise test and the findings (Table 1.2) show that there were no significant differences in $\%\dot{V}O_{2\,MAX}$ between relatively equally trained adult and young female athletes at T_{vent}.

Table 1.2. Comparison of $\dot{V}O_2$ at T_{vent} and T_{vent} as $\%\dot{V}O_{2\,MAX}$ in young and adult female athletes. Mean (S.D.) From Bunc and Heller (1993).

Event	Number of subjects	Age (years)	$\dot{V}O_2$ at T_{vent} ($ml \cdot kg^{-1} \cdot min^{-1}$)	T_{vent} $\%\dot{V}O_{2\,MAX}$
Long distance runners	12	24.2 (2.3)	56.9 (2.9)	85.1 (3.8)
	11	16.3 (0.9)	49.4 (3.1)	84.9 (2.7)
Middle distance runners	10	22.9 (2.8)	51.7 (3.2)	82.9 (2.1)
	16	16.6 (0.8)	46.5 (2.4)	82.6 (3.2)
Canoeists	7	21.1 (2.1)	40.0 (2.2)	79.9 (2.6)
	8	16.0 (0.9)	38.3 (2.4)	79.4 (3.0)

It has been observed that the hyperventilation that occurs during incremental exercise, and the LT do not represent identical biochemical events (Green et al., 1983) and parameters associated with changes in pulmonary minute ventilation do not always track changes in blood lactate concentration (Brooks, 1985). In addition, under certain conditions such as glycogen depletion, T_{vent} may be altered such that ventilatory indices are not reliable estimates of LT (Pfitzinger and Freedson, 1997b). The T_{vent} probably occurs at a time slightly later than the abrupt increase of lactic acid in the serum during exercise (Washington, 1989). Although, the bases of the relationship binding LT and T_{vent} are not clearly known, it has been shown that the LT and T_{vent} are well correlated (Davis et al., 1979; Ivy et al., 1980; Caiozzo et al., 1982) which provides justification for its use. Mocellin et al. (1991) has however recorded a lactate threshold of 78 ± $3.6\%\dot{V}O_{2\,MAX}$ which was substantially higher than the T_{vent} (53 ± $8.8\%\dot{V}O_{2\,MAX}$) in eleven boys of age 11-12 years.

Although it might be assumed that since children cannot sustain high intensity exercise aerobically they would be expected to demonstrate significantly higher relative anaerobic thresholds in comparison to adults. However there is little direct evidence to

support such a viewpoint.

1.2.4. Efficiency of Exercise

Work efficiency (η) is the ratio between external mechanical work performed, and the chemical energy required by the muscles to perform the work (Whipp and Wasserman, 1969). The chemical energy required to perform work is calculated from the steady state oxygen uptake $\dot{V}O_{2(SS)}$ and the energy equivalent derived from the non-protein RER. As some of the measured energy is essential to sustain tissues at rest and is not associated with the production of work this must be accounted for and the calculation of η takes on the form :

$$\text{Work efficiency} = \frac{\text{Mechanical power output}^*}{\text{Exercise metabolic rate - Resting metabolic rate}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

* When work is done during a known period of time, power units rather than work units are used in the numerator and denominator (Bar-Or, 1983).

It is not always appropriate to use this method of calculation in children as prior activity and movement during the procedure to measure resting metabolic rate are difficult to control. By making use of the first order relationship between $\dot{V}O_2$ and work rate i.e. the time course of the observed $\dot{V}O_2$ induced by a ramp work rate protocol in humans is considered to parallel the $\dot{V}O_{2(SS)}$ after a time delay of four to five $\dot{V}O_2$ time constants (τ) :

$$VO_2(t) = VO_{2(SS)} \cdot (t - \tau) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

When time (t) is much greater than τ, $VO_2(t)$ lags $VO_{2(SS)}$ by τ (Whipp, 1987). The time constant τ (tau) of an exponential curve is $[(1\text{-}e^{-1})x100]$ and is generally considered as a parameter of the rate of change in oxygen uptake in response to a change in exercise intensity.

It is thus possible to calculate η by calculating the exercising metabolic rate from the change in oxygen uptake ($\Delta\dot{V}O_2$) 4 time constants (~ 60 seconds) after the start of a ramp exercise protocol to a point at or below the work rate which elicits anaerobic energy production. The mechanical power output is the change in work rate (ΔWR) over the same time period from which $\Delta\dot{V}O_2$ is calculated. Thus η may be calculated as delta

efficiency (Δefficiency) using equation 4.

$$\Delta\text{efficiency (\%)} = \frac{\Delta WR(W)}{\Delta \dot{V}O_2(ml \cdot min^{-1})} \times \frac{60}{20.609} \times 100\% \quad \text{.....(4)}$$

Since the calculated Δefficiency is virtually unaffected by values of respiratory exchange ratio in this physiological range, an RER of 0.9 is assumed (Godfrey, 1974; Cooper et al., 1984) and for 1 ml of oxygen consumed this is equivalent to the utilisation of 20.609 joules of energy. As a power output of 1 W is 1 joule·second^{-1}, Δefficiency can be calculated using a simplified version of the above formula (Equation 5):-

$$\Delta\text{efficiency} = \frac{0.291}{\text{Slope}} \quad \text{.....(5)}$$

where slope is $\Delta \dot{V}O_2(L \cdot min^{-1})/\Delta WR(W)$ (Godfrey 1974).

Other researchers (Davis et al., 1982; Hughson and Inman, 1986a) have chosen to use an RER of 0.95 corresponding to an energy equivalent of 20.87 joules. As the RER changes throughout the ramp test it is considered that the use of this constant value (RER = 0.95) will only result in errors of approximately 0.5% over the metabolic range of the calculation (Davis et al., 1982).

The assumption of constant RER means that the calculated energy is directly proportional to oxygen consumption, therefore it is simpler to express the individual response to the ramp exercise protocol in terms of economy of work :

$$\text{Work economy} = \frac{\Delta \dot{V}O_2(ml \cdot min^{-1})}{\Delta WR(W)} \quad \text{.....(6)}$$

The O_2 cost of activities that involve running or walking have been found to be higher in children than in older individuals (Robinson, 1938; Astrand, 1952; Rowland et al., 1987; Ebbeling et al., 1992). This has been attributed to differences in stride frequency, inefficient running mechanics, substrate utilisation, soft tissue differences, surface area to body mass ratio and thermoregulatory differences between adults and children (Rowland, 1990).

The traditional view is that the work efficiency of cycling is relatively fixed

(~10 $ml \cdot min^{-1} \cdot W^{-1}$) for a given work task and is independent of training, age, or gender (Wasserman and Whipp, 1975; Astrand and Rodahl, 1986; Hansen et al., 1987); the exception to this is the higher oxygen cost to move heavier limbs in obese subjects (Wasserman and Whipp, 1975). This conclusion is supported in a more recent study (Springer et al., 1991), where Δefficiency measured using a ramp technique was not significantly different in adults and children (11.6 (± 1.3) $ml \cdot min^{-1} \cdot W^{-1}$ in children and 10.9 (±1.5) $ml \cdot min^{-1} \cdot W^{-1}$ in adults) and also by Rowland et al. (1990) who concludes that the efficiency of muscular contraction during exercise is comparable in pre- and post-pubertal subjects.

In opposition to this, studies have found child-adult differences using a step technique to measure oxygen cost (work economy). In a step change from unloaded pedalling to 80% AT (Armon et al., 1991), children were found to have a significantly greater oxygen cost whilst cycling compared to adults, 11.92 (± 1.12) $ml \cdot min^{-1} \cdot W^{-1}$ and 9.34 (± 1.77) $ml \cdot min^{-1} \cdot W^{-1}$ in children and adults respectively ($P < 0.01$). Similarly, calculations from the results reported by Turley and Wilmore (1997) show that the oxygen cost of cycling between 40 and 60 W is greater in 7 to 9 year old boys (12 $ml \cdot min^{-1} \cdot W^{-1}$) than in adult males of age range 19 – 26 years (8 $ml \cdot min^{-1} \cdot W^{-1}$). The values for young girls (of age range 7 – 9 years) and adult females (of age range 19 – 26 years) were 11.5 and 9 $ml \cdot min^{-1} \cdot W^{-1}$ respectively. Finally, Hebestreit et al., (1998) confirmed these adult-child differences in oxygen cost of cycling at 50% $\dot{V}O_{2\,PEAK}$.

It may be that some of the studies which report no difference in the oxygen cost of cycling between adults and children have expressed the oxygen cost relative to body mass rather than in relation to the work performed. For example, in the study of Cooper et al. (1985) when resting values are taken into account and corrections made for body mass it is possible to calculate average values for the oxygen cost of cycling. These calculations show that the younger children, of age range 7 – 10 years have an oxygen cost of 18.7 $ml \cdot min^{-1} W^{-1}$, compared to the older children (15 - 18 years) whose oxygen cost was on average 13.3 $ml \cdot min^{-1} W^{-1}$.

In conclusion there appears to be considerable evidence to support the findings of an

increased oxygen demand for non-weight bearing activities in children.

With reference to the Fick equation (Section 1.2.1 Equation 1) the higher oxygen cost of submaximal work in children could be related to higher cardiac output ($\dot{Q}$) or increased a-$\bar{v}O_2$ difference. In spite of the higher heart rates of children during exercise at a given submximal work rate or oxygen uptake, the majority of studies have reported that both boys and girls (Bar-Or et al., 1971; Katsuura, 1986; Turley and Wilmore, 1997) have a lower $\dot{Q}$ than adults at a given sub-maximal work rate or oxygen uptake. This lower $\dot{Q}$ is attributed to a lower stroke volume (SV) which is only partially compensated for by a higher heart rate. The lower SV at a particular sub-maximal work rate is closely associated with a smaller left ventricular mass in children of both sexes (Turley and Wilmore, 1997). It may also be associated with a smaller pre-load and a higher after-load in children. The smaller venous return of children would contribute to a reduced pre-load which would in turn reduce the SV in children compared to adults. Children have a higher total peripheral resistance (TPR) during exercise at a standard submaximal work load when compared to adults (Turley and Wilmore, 1997) which may represent a higher after-load, both these factors would reduce SV in children in addition to the reduced body size. Therefore it is unlikely that $\dot{Q}$ is the physiological factor responsible for the higher oxygen cost of submaximal work in children.

In spite of the higher TPR during standard submaximal exercise in children compared to adults, more of $\dot{Q}$ may be directed to the working muscle (Koch, 1980). This increase in muscle blood flow could account for the larger a-$\bar{v}O_2$ difference found in children (Katsuura, 1986; Turley and Wilmore, 1997). Other factors could be an enhanced oxygen dissociation from haemoglobin and the a-$\bar{v}O_2$ difference, at a given $\dot{V}O_2$, being nearer the child's maximum value since the children would have been exercising at a higher relative intensity. The exact mechanisms responsible for the higher a-$\bar{v}O_2$ difference is not known and is unlikely to be resolved easily because of the invasive nature of the research that would be required.

1.3. Summary

Since the work of Robinson (1938) significant progress been made towards the understanding of how the processes of growth and maturation impinge upon the child's chronic and acute responses to exercise. New techniques such as ^{31}P-MRS are now providing an insight into the metabolic control of cellular respiration (Zanconato et al., 1993; Taylor et al., 1997), information which in the future may provide the basis for exercise prescription in the healthy or help in the diagnosis and rehabilitation of the child with disease. A great deal of work has been carried out looking at the issues of scaling the child's physiologic response to exercise (Rowland, 1998), establishing suitable criteria for determining $\dot{V}O_{2\ MAX}$ (Rivera-Brown et al., 1992; Duncan et al., 1996), or in establishing non-invasive indices for the determination of the anaerobic threshold (Washington 1993; Chicharro et al., 1995; Pfitzinger and Freedson 1997b). All of these issues have yet to achieve a consensus. It may be considered that part of the reason for this may be a result of the practice of applying exercise protocols and models which have been designed for use in adults. These methods of investigation may not provide the most appropriate exercise stimuli for assessing the child's response to exercise.

A useful way of quantifying cardiorespiratory responses to exercise is to measure the metabolic response to exercise transitions, oxygen uptake kinetics ($\dot{V}O_{2\ KINETICS}$). The measurement of $\dot{V}O_{2\ KINETICS}$ may prove to be a useful tool in establishing new criteria for characterising exercise performance in children through a non-invasive technique, without the necessity to scale the response in relation to body size and without the requirement for children to exercise to exhaustion.

2. Oxygen Uptake Kinetics

In this chapter the background to the sub-maximal assessment of $\dot{V}O_{2\ KINETICS}$ in children is reviewed in 7 distinct sections. Sections 2.1 and 2.2 consider the definition of and the physiological basis for $\dot{V}O_{2\ KINETICS}$. Section 2.3 covers the issues relating to breath-by-breath assessments of oxygen uptake and serves as a precursor to Section 2.4 which relates to the process of measurement of $\dot{V}O_{2\ KINETICS}$. Section 2.5 reviews the literature relating to the physiological mechanisms responsible for controlling $\dot{V}O_{2\ KINETICS}$. The applications of the measurement of $\dot{V}O_{2\ KINETICS}$ are considered in Section 2.6 and finally Section 2.7 reviews the various studies which have described the measurement of oxygen uptake kinetics in children

2.1. Definitions

At the onset of exercise the immediate energy demands to fuel muscle contraction are met by the free energy of hydrolysis of adenosine triphosphate (ATP). The process of replenishing the local ATP pool, via creatine kinase-linked breakdown of phosphocreatine (PC), is an oxygen requiring mechanism taking place within the mitochondria and it is this demand for oxygen that eventually manifests itself as the increased oxygen utilisation measured at the mouth of an exercising individual.

Since the stores of oxygen in the body are very small relative to metabolic demand, the dynamics of $\dot{V}O_2$ measured at the mouth during exercise transitions are closely coupled to cellular events (Cooper et al., 1985). The measurement of $\dot{V}O_{2\ KINETICS}$ can therefore provide a useful insight into the mechanisms of cellular energy production.

At the onset of a step increase in exercise intensity the oxidative processes that provide energy for the resynthesis of ATP cannot match the demand for ATP. Blood oxygen stores (oxyhaemoglobin in venous blood), physically dissolved oxygen and oxyhaemoglobin in the muscles contribute little to the overall energy supply, and therefore energy must be supplied by other sources, for example the depletion of muscle PC and the anaerobic metabolism of pyruvate. The difference between the total oxygen

that would have been consumed, to meet the energy demands of the contracting muscle, and the actual oxygen utilised, is known as the oxygen deficit. The oxygen deficit is therefore inversely related to $\dot{V}O_{2\,KINETICS}$. Individuals with rapid $\dot{V}O_{2\,KINETICS}$ rely less on anaerobiosis at the start of exercise to supplement energy requirements than those with slower $\dot{V}O_{2\,KINETICS}$ (Whipp and Wasserman, 1986).

There are a number of exercise transitions which have been used experimentally to stimulate cellular energy production in investigations relating to $\dot{V}O_{2\,KINETICS}$. The five most useful are the step (Hebestreit et al., 1998), impulse (Fujihara et al., 1973), ramp (Cooper et al., 1984), sinusoid (Haouzi et al., 1993) and the pseudo-random binary sequence (PRBS) (Hughson et al., 1990a) work rate perturbations.

The step change in intensity is the simplest of these tests to administer and the resultant characteristic three phase $\dot{V}O_2$ response (Linnarsson, 1974; Whipp et al., 1982) described below (Figure 2.1) can be easily identified.

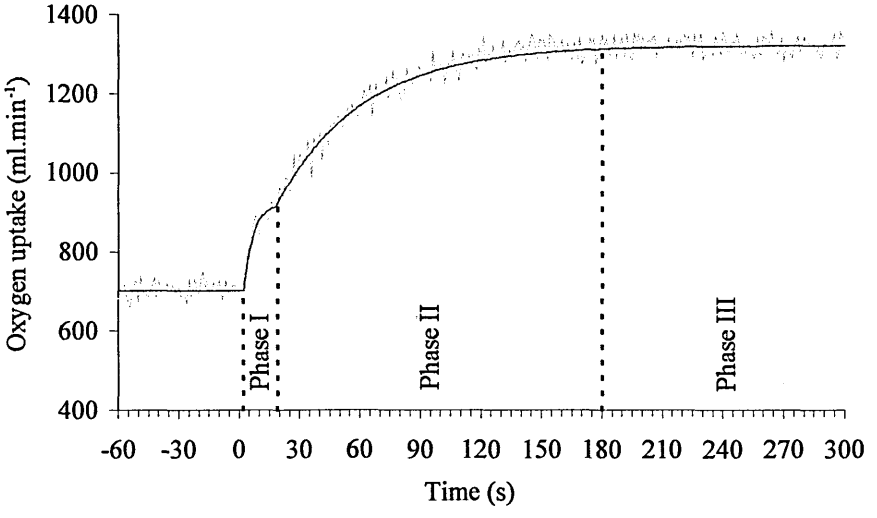


Figure 2.1. The 3 phases of oxygen uptake increase in response to a step increase in external work below the anaerobic threshold. Time 0 indicates onset of 80 W cycling from a baseline of 20W. To enhance the underlying response, the data from seven identical tests have been superimposed and the average response calculated.

The initial exercise stimulus results in an immediate increase in cardiac output ($\dot{Q}$), pulmonary blood flow and a consequent abrupt increase in $\dot{V}O_2$ which can be identified as **Phase I** of the response described in Figure 2.1. During this phase the venous blood from the active muscle has *not* yet reached the lungs and the increase in $\dot{V}O_2$ is

dependant on the magnitude of the change in work rate and does not reflect muscle oxygen utilisation. As Phase I is not temporally related to cellular metabolism, many researchers design protocols in an attempt to minimise the Phase I response by employing work-to-work, rather than rest to work, exercise transitions.

Phase I lasts approximately 15 to 20 s and is succeeded by **Phase II**, at which point the venous blood from the active muscles arrives at the lungs. The Phase II response is characterised by an exponential increase in $\dot{V}O_2$ (should the metabolic demand not have already been satisfied by the increased oxygen uptake during Phase I). The reduced oxygen content of the venous blood leaving the active muscles reflects the increased oxygen required to sustain muscular activity at the desired intensity. Oxygen uptake continues to rise until the oxygen demands of the active muscles are met, or the oxidative processes are operating at full capacity. If all the energy requirements are able to be met by aerobic metabolism then **Phase III** of the response is a plateau or *steady state* and the exercise is described as being of either *moderate* or *low intensity*. Phase III (steady state) is reached after approximately 3 minutes of low or moderate intensity exercise and, in normal adults, the relationship between the increase in $\dot{V}O_2$ and the increase in work is approximately 10 $ml.min^{-1}.W^{-1}$ at constant pedal frequency (Hansen et al., 1984). This value of 10 $ml.min^{-1}.W^{-1}$ is considered to be independent of factors such as fitness (Whipp and Wasserman, 1972) but may be influenced by body weight (Wasserman and Whipp, 1975) gender (Astrand, 1960) and maturation status (Hebestreit et al., 1998). In conditions of high intensity exercise, oxidative processes alone are insufficient to sustain the required level of work and cellular energy production is supplemented by anaerobic metabolism, in which case Phase II no longer terminates (after approx. 3 minutes of exercise) in a steady state Phase III plateau, but is extended and takes the form of a gradual, linear increase in the rate of oxygen utilisation (Wasserman et al., 1967). This additional increment in $\dot{V}O_2$ is described as excess $\dot{V}O_2$ ($\dot{V}O_{2(xs)}$) and is a result of a *slow component* of the $\dot{V}O_2$ KINETICS which is superimposed upon the early $\dot{V}O_2$ response (Whipp, 1994). The $\dot{V}O_{2(xs)}$ is associated with exercise intensities which bring about an increase in blood lactate concentration

[lactate]. Oxygen uptake can attain a steady state (Phase III) above the lactate threshold (LT) but only in the work-rate range where lactate remains constant at an elevated level (Whipp, 1994). The highest sustainable level of $\dot{V}O_2$ has been demonstrated to coincide with the work rate at which blood [lactate] can no longer be maintained or perhaps falls slightly as the exercise continues and for arterial blood pH to be maintained at a constant, although lowered level (Poole et al., 1988). Figure 2.2 shows the $\dot{V}O_2$ responses to seven different work rates for a healthy subject (Casaburi et al., 1989). The lowest 3 work rates are below the subject's lactic acidosis threshold, whereas the 4 highest work rates are above it. The $\dot{V}O_2$ continues to rise for the 4 work rates above the lactic acidosis threshold, the rate of rise being more marked the higher the work rate.

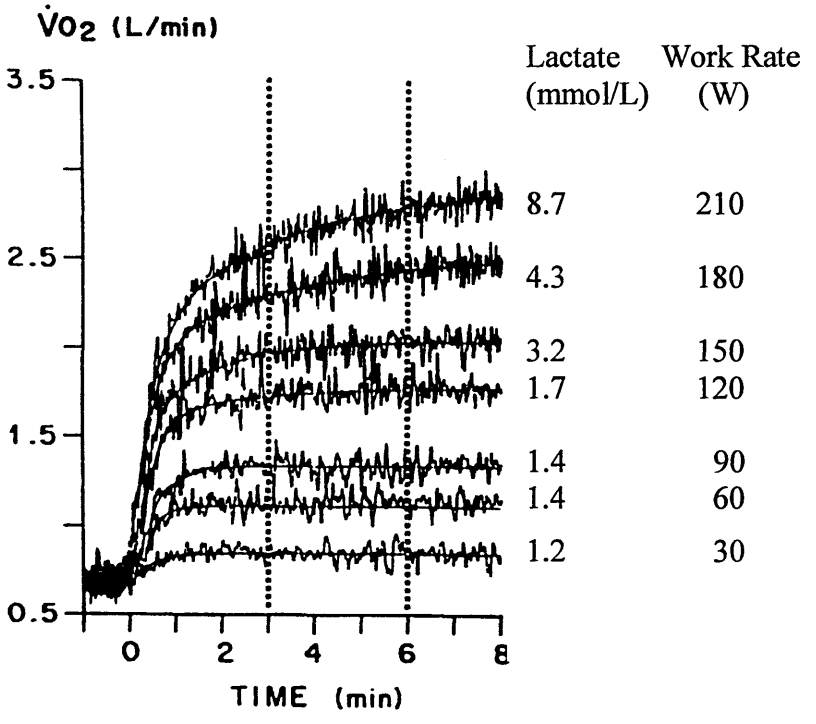


Figure 2.2. Second-by-second responses of a subject undertaking exercise transitions between unloaded pedalling and each of 7 higher work rates (Casaburi et al., 1989).

2.2. Physiological Basis of Oxygen Uptake Kinetics

The characteristic three phase change in oxygen uptake in response to a step increase in work rate is the result of an integrated cardiovascular, respiratory and cellular adjustment in order to supply oxygen for muscular activity. These adjustments are not

only required to support the increased mitochondrial O_2 utilisation in the active muscles, but also to regulate the resultant arterial blood-gas and acid-base status during exercise.

2.2.1. Cardiovascular and Respiratory Responses at the Onset of Exercise

The cardiovascular and respiratory responses at the onset of exercise are initially under neural control which simultaneously increases blood flow and initiates increases in gas exchange at the lung. Subsequent mechanical and metabolic effects also increase the blood flow and therefore the oxygen supply to the working muscles. Krogh and Lindhard (1913) were first to describe the initial neural response to exercise as involving an irradiation of impulses from the motor cortex, and sensory information from the proprioreceptors to the respiratory centres in the brain. This mechanism increases the activity of the autonomic respiratory centre and results in an increased rate of pulmonary ventilation ($\dot{V}E$). In a study using a walking protocol D'Angelo and Torelli (1971) concluded that the initial rapid changes in $\dot{V}E$ at the onset of exercise was independent of the partial pressure of CO_2 in the lungs and could be attributed to a neural mechanism. By introducing hypocapnic conditions during cycle exercise Cerretelli et al. (1995) also demonstrated that the initial ventilatory adjustment to exercise was likely to be of neural origin and supported the hypothesis that the impulses arising from the mechanoreceptors in exercising muscles and joints produced an 'exercise reflex'.

After the initial neurally mediated increase in $\dot{V}E$, the parasympathetic influence on the heart is reduced and sympathetic stimulation increased, with a resultant increase in the rate and force of contraction of the heart. Hughson (1990) described the control of heart rate in response to light exercise as being mainly based on parasympathetic withdrawal, with sympathetic activity being increased at heart rates above 100 beats·min^{-1}.

Adaptations in blood flow also occur as a result of sympathetic stimulation of the vasculature. Initially, blood flow is reduced to the nonessential organs as a result of peripheral vasoconstriction. Sympathetic neural impulses initiate vasodilation of the central blood vessels providing an increased delivery of oxygen to the exercising

muscles (D'Angelo and Torreli, 1971; Wasserman, 1982). Venoconstriction results in a reduced blood volume held in the venous side of the circulatory system and additionally, venous return and therefore stroke volume, is increased by the physical pumping action of exercising muscle. These circulatory changes result in a decrease in the pressure gradient in the veins draining the muscle bed which, in turn, lead to a greater pressure gradient across the muscle bed and increased blood flow to the exercising muscles.

Changes in the concentration of metabolites, for example increases in adenosine, potassium ions, hydrogen ions, osmolarity and decreases in partial pressure of oxygen (PO_2), cause vasodilation in the arterioles of the active skeletal muscle. These changes however, do not fully explain the hyperaemia which must also involve dilation of the feed arteries to the active muscle. Nitric oxide released by endothelial cells has been implicated as a mediator in this response.

The overall effect of these cardiovascular and respiratory responses at the onset of exercise is to increase the $\dot{Q}$ and redistribute the flow of blood into the active muscle bed.

2.2.2. Oxygen Utilisation by the Muscle

The free energy for all cell function and maintenance appears to be provided by the hydrolysis of ATP. The ultimate source of almost all ATP produced in the muscle is oxidative metabolism with PC breakdown providing virtually instantaneous replenishment of ATP. In conditions of even dramatic increases of metabolic rate controlling mechanisms act in order to maintain nearly constant cellular ATP (Meyer and Foley, 1996).

In response to exercise, ATP production in skeletal muscle is increased via both feed forward and feed backward mechanisms. Calcium (Ca^{++}) released from the sarcoplasmic reticulum acts in a feed forward manner to activate glycolysis and oxidative phosphorylation. The increased ADP/ATP ratio which occurs as a result of muscle contraction acts in a feedback manner to stimulate additional ATP production

(Meyer and Foley, 1996). The key roles of Ca^{++} and phosphate metabolites in regulating ATP production are schematically outlined in Figure 2.3.

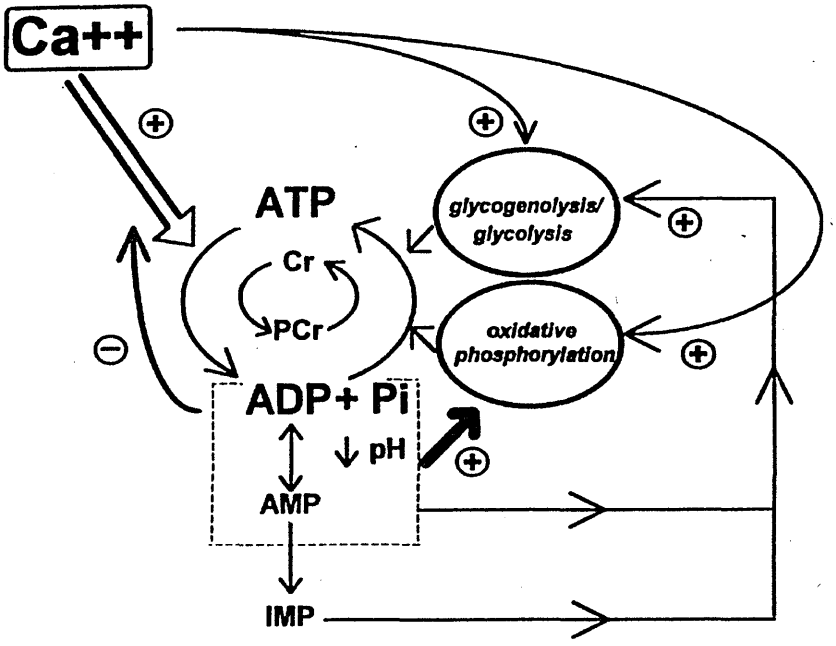


Figure 2.3. Schematic illustrating the key roles of calcium and phosphate metabolites in regulating force and ATP production in skeletal muscle (Meyer and Foley, 1996). ATP = Adenosine tri-phosphate, ADP = Adenosine mono-phosphate and AMP = Adenosine mono-phosphate. Pi = inorganic phosphate. Cr = creatine and PCr = phosphocreatine. IMP = inosine mono-phosphate.

It is unlikely, however, that muscle respiration is under the simple control of cytoplasmic [ADP]. In the cytoplasm there may also be other potential linking regulators such as inorganic phosphate and adenosine mono-phosphate (AMP) or the phosphorylation potential [ATP]/([ADP] x [inorganic phosphate]) in the mitochondria. Some studies have shown that respiratory rate depends upon intramitochondrial [NADH]/[NAD] ratio as well as extramitochondrial nucleotides and phosphate.

Although the exact nature of the control mechanisms coupling the rate of hydrolysis with the rate of oxidative phosphorylation remain controversial, mitochondrial creatine kinase (CK) appears to play a pivotal role, since the proportional exponential increase in muscle $\dot{V}O_2$ parallels the decrease in levels of PC (McCreary et al. 1996). In spite of the complex nature of the system coupling muscle oxygen utilisation with ATP

hydrolysis, the process has been shown to have the response of a simple linear, first order system (Mahler, 1985) and it behaves as if it is limited by a single rate limiting step. There remains some controversy surrounding the mechanism(s) responsible for the rate limiting factor for $\dot{V}O_{2\,KINETICS}$ (Tschakovsky and Hughson, 1999). It is likely that the site is at the cellular level as described by Mahler (1985) and (Yoshida and Whipp, 1994). The evidence for and against the peripheral limitation of $\dot{V}O_{2\,KINETICS}$ is discussed more fully later in this chapter (Section 2.5).

2.3. Methods of Measuring Oxygen Uptake

2.3.1. Open Circuit Spirometry

An assessment of energy expenditure in humans may be made by the direct measurement of heat generation, a method referred to as 'calorimetry'. As energy is produced in the human body by means of chemical reactions, it is possible to evaluate energy expenditure indirectly from the measurements of the substances consumed and the products formed. Energy expenditure may therefore be determined from the amounts of oxygen consumed ($\dot{V}O_2$) and carbon dioxide produced ($\dot{V}CO_2$) (Consolazio et al., 1963). This principle forms the basis for the investigation of energy expenditure in humans using respiratory gas analysis.

There are two methods of respiratory data collection, 'closed' and 'open' circuit spirometry. In closed-circuit spirometry the subject breathes from a pre-filled container or spirometer of oxygen. This type of spirometry has specialist applications, for example, investigations into the effects of the low oxygen tensions experienced at altitude, and clinically in the diagnosis of cardio-respiratory disease. Open-circuit spirometry, whereby the subject inspires from the atmosphere, provides a convenient and (under defined conditions) a valid and accurate method for measuring oxygen consumption and carbon dioxide production during both rest and exercise (Consolazio et al., 1963). The calculation principle for the determination of oxygen consumption using open-circuit spirometry is essentially the same process whether gas collection is performed manually using Douglas Bags, or automatically using a computerised

system.

Oxygen uptake (VO_2) is calculated as the difference between the quantity of oxygen inhaled and exhaled:-

$$VO_2 = VIO_2 - VEO_2 \quad \text{.....(7)}$$

where VIO_2 and VEO_2 are the volume of oxygen inspired and expired respectively. VO_2 is normally expressed as a rate of oxygen utilisation per minute and is symbolised as $\dot{V}O_2$.

Expired air is collected over a known period and its volume measured. A sample of the expirate is then dried and passed through both an oxygen and a carbon dioxide analyser to determine the O_2 and CO_2 composition of the expired air. The results of the analysis are expressed as a fraction of the total mixed expired gas volume ($F\bar{E}O_2$ and $F\bar{E}CO_2$). VEO_2 is the product of the volume (VE) and the fractional mixed concentration of oxygen ($F\bar{E}O_2$) in expired air.

$$VEO_2 = VE \times F\bar{E}O_2 \quad \text{.....(8)}$$

When using open-circuit spirometry the volume of inspired air (VI) is not usually measured, however by using a principle known as the Haldane transformation, VI may be determined from VE. The assumption used in the Haldane transformation is that nitrogen is physiologically inert (Wilmore and Costill, 1973a), and on average the amount of nitrogen inspired (VIN_2) is said to be equal to the amount expired (VEN_2) or;

$$VIN_2 = VEN_2 \equiv VI \times FIN_2 = VE \times F\bar{E}N_2 \quad \text{.....(9)}$$

where FIN_2 and $F\bar{E}N_2$ are fractions of inspired and mixed expired nitrogen respectively.

By rearranging equation (9)

$$VI = VE \times \frac{F\bar{E}N_2}{FIN_2} \quad \text{.....(10)}$$

VI differs from VE in direct proportion to the ratio of inspired to expired nitrogen concentration (equation 10 - the Haldane transformation).

$F\bar{E}N_2$ is calculated by subtraction :-

$$F\bar{E}N_2 = 1-(F\bar{E}O_2 + F\bar{E}CO_2) \quad \text{.....(11)}$$

FIN_2 is 0.7904 i.e. the fractional composition of nitrogen in dry ambient air. It is

important to note that the quantity of N_2 present in the inspired and expired air collected over a period of at least 20-30 seconds is assumed to be constant (Zebballos and Weisman, 1994). Any difference in $F\bar{E}N_2$ and FIN_2 is due to the differences in VI and VE brought about by CO_2 and O_2 being produced and consumed at different rates.

FIO_2 is taken to be 0.2093 (the fractional composition of oxygen in dry ambient air) and VIO_2 may be calculated as:-

$$VIO_2 = FIO_2 \times VI \quad \text{.....(12)}$$

VO_2 may now be calculated by inserting the values for VEO_2 and VIO_2 into equation (7).

For the calculation for VCO_2 it is often accepted that the volume of carbon dioxide inspired ($VICO_2$) is small enough to be considered negligible, so that :

$$VCO_2 = VECO_2 \times VE \quad \text{.....(13)}$$

2.3.2. Automated Gas Analysis Systems

The first fully automated gas analysis system with the capacity to measure oxygen uptake on a breath-by-breath basis was described by Auchincloss et al. (1966). Respiratory gas analysis, like many other systems developed in the 1960's and early 70's, benefited from the application of computer technology and were refined by the development of rapidly responding electronic gas analysers and flow/volume sensing devices. The first fully automated, computerised data collection and analysis systems were designed around existing computing facilities such as the mini-computer based system described by Whipp and Wasserman (1972) and Beaver et al. (1973). Computing systems of the 1970's were, however, relatively large and very expensive and as such the availability of computing power was perhaps the governing factor in early gas analysis system development. In response to the limited computing time available the approach of Pearce, et al. (1977) was to separate data collection from analysis by using an FM tape recorder as an intermediate storage facility. Computer analysis would then be carried out post-test, thus not restricting testing to the availability of a computer terminal. Other systems developed over the same time period

Table 2.1 Development of Automated Gas Analysis Systems (*See text for explanation).

	i) volume measurement		ii) gas analysis,	iii) integration of signals	iv) data resolution
AUTHOR	MIXING BOX	VOLUME / FLOW DEVICE	ANALYSER(S)	ALIGNMENT	REPORTING INTERVAL
Kissen & McGuire (1967)	No	Linear mass flow meter.	O_2 –Beckman 7841IV	Not described.	30 seconds
Whipp & Wasserman (1972)	No	Pneumotachometer and strain gauge.	O_2 –Westinghouse 211 CO_2 –Beckman LB-1	Described*	Breath-by-breath
Beaver et al. (1973)	No	Pneumotachometer and differential pressure transducer.	O_2 –Westinghouse 211 CO_2 –Beckman LB-1	Described*	Breath-by-breath
Wilmore & Costill (1973b)	Yes	Pneumotachometer or Gas meter	O_2 –Beckman E2 CO_2 –Beckman LB-1	Not applicable	10 seconds
Wilmore et al. (1976)	Yes	Volume transducer turbine with bias airflow.	O_2 –Beckman OM-11 CO_2 –Beckman LB-2	Not applicable	30 second
Pearce et al. (1977)	No	Pneumotachometer and pressure transducer.	O_2 –Thermox fuel cell CO_2 –Beckman LB-1	Described*	Breath-by-breath
Sue et al. (1980)	No	Pneumotachometer and differential pressure transducer.	O_2 –Applied Electrochemistry S3-A CO_2 –Beckman LB-2	Described*	20 second

were designed in order to minimise the amount of computing hardware used. For example, the semi-automated system described by Wilmore and Costill (1973b) and the system of Sue et al. (1980) employed programmable calculators to co-ordinate, manipulate and store data for later use.

Table 2.1 provides a description of the varied approaches to the automation of respiratory gas analysis between the years of 1967-1980. Each of the column headings in Table 2.1 shows either a major physical or methodological variable.

The basic components of any automated respiratory analysis system may be broken down into four main areas: i) volume measurement, ii) gas analysis, iii) integration of signals, and iv) data resolution.

2.3.2.1. Volume Measurement

There are two main methods of determining the volume of respiratory gases:

1) The expirate is collected in a "mixing box" over a defined time period and its volume measured using a spirometer (Sue et al., 1980) or dry gas meter (Wilmore and Costill, 1973b).

2) The volume is measured indirectly as the integral of flow against time using either a pneumotachometer or a turbine.

A pneumotachometer is a device which uses a differential pressure transducer in order to calculate flow. Prior to 1980, pneumotachometers used in respiratory gas analysis tended to be unidirectional and were used only for the determination of expired volumes.

Both the pneumotachometer and the turbine presented the manufacturers with their own unique problems in terms of accuracy and reliability. The devices produced were required to accurately measure pulsatile flow rates of 5 $l \cdot min^{-1}$ to over 200 $l \cdot min^{-1}$, over a range of temperatures, water vapour saturation and air viscosities.

A particular problem encountered in the use of turbine devices was the inertial resistance of the rotating blade. The designers of flow measurement devices employed various techniques in order to account for these factors. The turbine used by Wilmore et

al. (1976) was kept constantly rotating by a bias airflow thus reducing the inertial resistance of the blade. The bias airflow restricted flow measurement to one direction. Accurate measurements of flow rate using a pneumotachometer require information about the flow-pressure characteristics over the full range of flow rates, obtained under the circumstances in which it is intended operate (Finucane et al., 1972). Technically the design of the pneumotachometers is fairly complex and as such are subject to error due to a variety of conditions such as the geometry and size of the system.

2.3.2.2. Gas Analysis

Table 2.1 also shows the range of gas analysers used by each of the early systems. Again the type of analyser used presented its own particular problems, the analysers used by Whipp and Wasserman (1972) measured the partial pressures of both inspired and expired CO_2 and O_2, and are affected by the variability in the partial pressure of water vapour of the inspired gas. To minimise this variability the inspired gas passed through a trough containing water at body temperature. It was also recognised by Kissen and McGuire (1967), that their polarographic device was subject to the effects of not only water vapour but also to fluctuations in barometric pressure and temperature, it was suggested that electronic compensation for these discrepancies could be achieved fairly easily. The Beckman OM-11 oxygen analyser (Wilmore et al, 1976) did show some attenuation attributed to water condensation, however this accounted for less than a 0.07% oxygen decrease on average and was not thought to grossly effect system accuracy. The response speed of the analysers shown in Table 2.1, with the exception of the Kissen and McGuire (1967) system, are reported as "fast responding" i.e. within 1 second.

During this time mass spectrometry was an option available for gas analysis but the sheer bulk and prohibitive cost of the mass spectrometer may have excluded its use in many laboratories. The use of the mass spectrometer as a reference system in evaluation studies (Wilmore and Costill, 1973b; Wilmore et al., 1976 and later by Jones, 1984) indicates its acceptance as an accurate method for gas analysis.

2.3.2.3. Integration of Signals

There are four main factors to be considered when designing systems for digital computation of oxygen uptake. The alignment of the ventilation signal with the measurement of fractional gas concentration, the sample rate of the input signal, and the clarity of the signal (noise) and the method of integrating the signals.

Alignment of ventilation with fractional gas concentration is necessary due to the differences in the way in which the two signals are generated. The volume measurement device produces its signal almost instantaneously. The signal from the gas analyser(s) is delayed by the time required to transport the gas to the analysers and the response time of the individual analysers. The time delay between the two signals is often referred to as the lag time, and once determined is used to match the time course of each volume excursion with the time course of its corresponding fractional gas concentration.

In the four examples in Table 2.1 where signal alignment is described, the lag time is taken as sample transport time (Beaver et al., 1973; Pearce et al., 1977), sample transport time plus unspecified response characteristics of both analysers (Whipp and Wasserman, 1972) and sample transport time plus specified analyser response time (Sue et al., 1980). Each different approach to the determination of lag time will lead to discrepancies in oxygen uptake determination. A computer simulation (Bernard, 1977) was used to investigate the effects of altering the lag time on the calculation of pulmonary gas transfer and concluded that, to be within 5% of the true value, the lag time should be accurate to ± 25 ms. This finding is also supported by the work of Hughson et al. (1989) using an experimental design.

The sample rate of the input signal must be set at the appropriate level and any noise associated with the instrumentation removed in order to maintain accuracy. Bernard (1977) concludes that, regardless of the filtering technique, sample rates of 30 Hz or more gave calculated values which were within 5% of the exact value (assuming that the noise is not close to the sampling frequency or its harmonics) suggesting that at high enough sampling rates the use of filtering techniques is unnecessary.

Depending upon the method of calculation, the shape of the expiratory flow pattern may

also have a significant effect on the calculation of true oxygen uptake. There are two methods of calculation when sampling is performed continuously, either the VE and FEO_2 signals are time aligned and integrated simultaneously, or, to avoid the process of time alignment, the VE and FEO_2 signals can be integrated separately and the mean mixed $F\bar{E}O_2$ calculated and multiplied by tidal volume for each breath. The second method is an approximation which uses a time-weighted average $F\bar{E}O_2$ instead of a volume-weighted value. The result can lead to errors which are dependent on the shape of the expiratory curve (Bernard, 1977).

2.3.2.4. Data Resolution

Data resolution refers to the reporting interval between measurements i.e. whether the system is capable of breath-by-breath analysis, or if it is limited by the minimum time span for which results could be generated. The reporting interval, which will be dependant on the response of the analysers, will have significant effect upon the system's application. Any system which has analysers unable to response within 100ms, are unlikely to generate data on a breath-by-breath basis and will not be suitable for studies of $\dot{V}O_{2\,KINETICS}$.

In conclusion, the specifications of the systems described in Table 2.1 differ in many respects. Some differences are a result of the purpose of their design. The systems of Whipp & Wasserman (1972), Beaver et al. (1973) and Pearce et al. (1977) were designed to study the kinetics of transient ventilatory and metabolic responses, whereas the systems described by Wilmore & Costill (1973b), Wilmore et al. (1976) and Sue et al. (1980) were designed to be mobile and less reliant upon major computer installations. The effect of these varied approaches will result in differences in the actual measurement of $\dot{V}O_2$ and $\dot{V}CO_2$.

2.3.3. Breath-by-Breath Methodology

The method of calculation used by manual and automated systems which measure VE, $FECO_2$ and FEO_2 relies upon the Haldane transformation for the calculation of VI (Equation 10). On a breath-by-breath basis VIN_2 and VEN_2 will not be equal since the

volume of the inspired breath is unlikely to be the same as the volume expired. The Haldane method calculates gas exchange at the mouth, which for a number of breaths grouped together accurately represents mean gas transport. Gas transport for individual breaths determined in this manner can however, can be quite inaccurate (Auchincloss et al. 1966; Beaver et al., 1981). It is important to avoid these discrepancies when studying oxygen transients as each breath, measured in response to the work transition, contains significant information.

Not only do normal physiological variations result in unequal magnitudes of inspiratory and expiratory tidal volume, they are also associated with changes in lung fractional gas concentrations on a breath-by-breath basis. As a consequence, lung gas stores (of oxygen, carbon dioxide and nitrogen) vary breath-by-breath. This must be accounted for to yield a better estimate of alveolar gas exchange which in turn better reflects oxygen uptake at the muscle during exercise (Barstow et al., 1994).

Beaver et al. (1981) have proposed a method to estimate alveolar gas exchange which relies upon the respiratory analysis system being able to measure both VI and VE and requires a gas analysis system able to determine nitrogen concentration e.g. a mass spectrometer. This method uses an estimate of nominal lung volume (NLV) which represents the area of the lung involved in gaseous exchange. Since NLV is not a measure of anything physical, functional residual capacity (FRC) is used as the equivalent to NLV. The value of NLV does not affect the mean of the $\dot{V}O_2$ and $\dot{V}CO_2$ estimates significantly but can significantly reduce the breath-to-breath variation, which is typical of the analysis when measuring $\dot{V}O_2$ at the mouth using the Haldane method. Functional residual capacity can be estimated from age, height, weight and sex using nomograms (Taylor et al., 1989).

The calculation for breath-by-breath alveolar gas exchange for oxygen uptake, using the Beaver et al. (1981) algorithm is :

$$\dot{V}O_2 = [(VIO_2 - VEO_2) - (\Delta VL \times FetO_2) - (\Delta FetO_2 \times NLV)] \times fb \quad \text{.....(14)}$$

where fb is the frequency of breathing (respiratory rate) in breaths per minute and,

$$\Delta VL = [(VN_2 - NLV) \times \Delta FetN_2] / FetN_2 \quad \text{.....(15)}$$

ΔVL represents the change in lung volume on a breath by breath basis (the breath-by-breath equivalent of the Haldane transformation - Equation 10)

On a breath-by-breath basis VN_2 will fluctuate about a mean of zero and is calculated in the same manner as VO_2 (Equation 7) i.e. :

$VN_2 = VIN_2 - VEN_2$ .....(16)

A positive VN_2 means that the inspired volume was greater than the volume expired. A negative value means the opposite (relatively larger expiration than inspiration).

The changes in fractional concentration (ΔFet) of N_2 and O_2 are estimated from changes in end tidal gas concentrations :

$\Delta FetN_2 = FetN_2 - FetpN_2$ .....(17)

$\Delta FetO_2 = FetO_2 - FetpO_2$ (where p = previous breath) .....(18)

$FetO_2$ and $FetN_2$ are the end tidal fractional concentrations of oxygen and nitrogen respectively.

Figure 2.4 shows the difference in breath-to-breath variability between calculating oxygen uptake on a breath-by-breath basis using the traditional Haldane method, by direct measurement of both inspired and expired oxygen concentration and volumes, and by applying the algorithm of Beaver et al. (1981). Note the differences in breath-to-breath variability between the three calculation methods.

2.3.3.1. Minimising Breath-by-Breath Variability

The lowest breath-to-breath variation yields the best estimate of gas exchange at the alveolar-capillary level (Swanson, 1980). The best method to minimise the random variation in breath-by-breath data is however equivocal (Hughson and Swanson, 1989). In order to account for the breath-to-breath changes in nominal alveolar volume Swanson (1980) has described an effective lung volume (EVL) which is calculated post-test as part of a general linear model that attempts to minimise the residual sum of squared error between the data and a model of best fit. Based on these post hoc calculations Hughson (personal communication) recommends the use of the algorithm of Beaver et al. (1981) but with an NLV of approximately ½ the FRC. This provides a

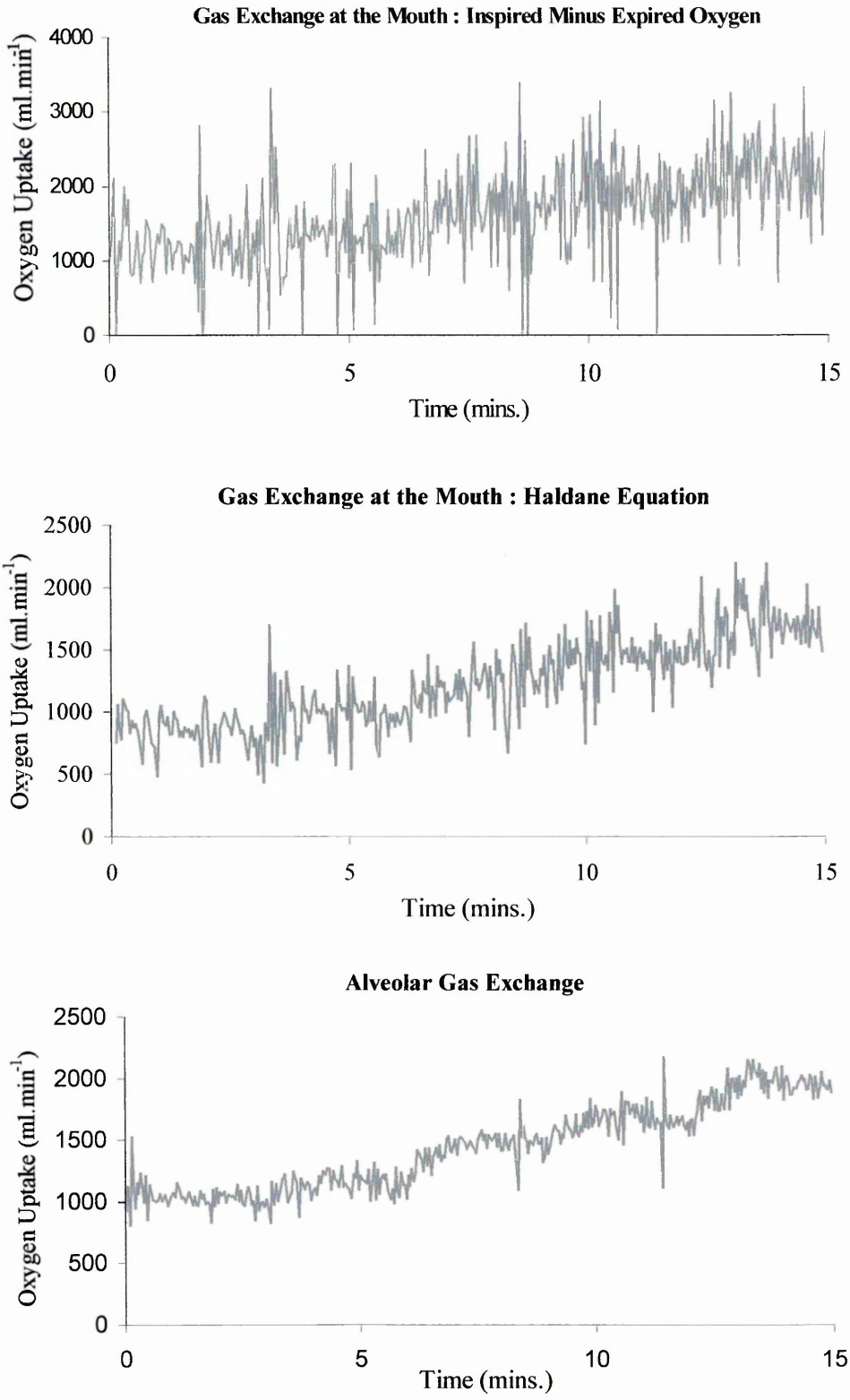


Figure 2.4. Breath-by-breath oxygen uptake calculated using the traditional Haldane transformation method, equations 7 - 12 (top figure). Breath-by-breath oxygen uptake calculated from the direct measurement of inspired and expired oxygen volumes, equation 7 (middle figure), and using estimated alveolar gas exchange equations 14 - 18 (bottom figure). Data collected during an incremental cycle ergometer exercise test (25 – 150 W in five, 3-min, 25 W increments).

reasonable estimate of ELV but without the complexity of post-test calculations. To simplify the calculation of alveolar gas exchange further, instead of measuring FRC, Hughson et al. (1991a) considered that, due to the relatively small difference between using an estimate and the measured value of FRC on minimising the breath-to-breath variability, it is appropriate to predict FRC based on height, age and sex using the reference data from Taylor et al. (1989).

2.3.4. Quality Control

Owing to the lack of a standard specification or recommendations for calibration and operation of respiratory analysis equipment, differences in system design may result in different measurement characteristics. There is a need to establish a suitable standard for comparison and evaluation of any new system, particularly in view of the fact that the traditional method for comparison, the Douglas Bag, does not have the resolution necessary to measure oxygen uptake on a breath-by-breath basis.

One of the main considerations with both the Douglas bag and the automated methods of respiratory gas determination are associated with accurate calibration of a) the volume measurement device and b) the gas analysers.

2.3.4.1. Volume Calibration

A high accuracy water filled spirometer such as the Tissot spirometer is recommended by the British Association of Sport and Exercise Sciences (BASES) as the reference standard for assessing gas volumes (personal communication) but is expensive, large and cumbersome. A commonly used alternative is the Harvard dry gas meter which uses an optical sensor to measure volume. The Harvard system is sensitive to changes in flow rate and care is required during use and calibration to provide a constant airflow using a vacuum pump.

Automated breath-by-breath systems are calibrated using a 1L or 3L calibration syringe. The method of calibration, whether using a pneumotachometer or a turbine, is critical to the measurement and should account for the full range of pulsatile flow rates expected during the measurement procedure. A mechanical device which can artificially simulate

respiratory gas exchange, such as the Gas Exchange Calibrator (Huszczuk, et al. 1990), is available but is expensive and does not simulate the natural variation in respiratory variables such as VE and VO_2 which occur on a breath-by-breath basis. The calibration of the syringes and dry gas meters should be checked on a periodic basis using a water filled reference spirometer.

The accuracy of most continuously monitoring volume measurement devices currently used in automated systems is usually to within 1%. The specification of the system is provided by the instrument manufacturers. An independent evaluation of volume measurement devices can be commissioned by the American Thoracic Society (ATS) and many flow / volume instruments carry the ATS certification. There is no comparative standard for British systems.

2.3.4.2. Gas Analyser Calibration

If skilful technical support is available, the Haldane, Scholander or Van Slyke volumetric methods are extremely good methods of gas analysis (Kissen and McGuire, 1967). The British Association of Sport and Exercise Science, as part of its accreditation procedure, have used the Scholander (1947) method as a reference for assessing the accuracy of commercially available gases. This method uses volumetric changes brought about by the absorption of oxygen by pyrogallol and carbon dioxide by potassium hydroxide, the absorbents are mixed with the gas by manually raising and lowering reservoirs. The end point is determined visually and the accuracy of the technique is dependent upon the apparatus being air tight. This method of gas analysis is unlikely to surpass the accuracy of commercially available gases which may now be purchased at very high tolerances i.e. to within ± 0.03 volume percent. Mass spectrometry and/or gravitational techniques are used by commercial suppliers to produce the low tolerances.

2.3.4.3. Standard for Comparison

The steady state oxygen consumption ($\dot{V}O_{2(ss)}$) of normal subjects is said to be predictable for cycle ergometer work, regardless of age, gender or training, with the

slope of the linear relationship between $\dot{V}O_2$ and work rate of approximately 10.1 $ml \cdot min^{-1} \cdot W^{-1}$ (Hansen et al., 1987). Whilst the efficacy of using the physiological responses to exercise as a calibrant has produced some debate (personal communication BASES Workshop), for many laboratories it is often the only viable quality control method available for assessing standards of both manual and automated gas analysis systems.

The American College of Sports Medicine (ACSM) (1991), Åstrand and Rodahl (1986) and Wasserman et al. (1987) have independently produced normative values for oxygen uptake response to cycle ergometer exercise (Figure 2.5). The body weight of the subject, cycling ergonomics and the physiological and psychological status of the biological calibrant as well as environmental factors may influence the prediction of $\dot{V}O_{2(ss)}$ responses to standard work loads. The 3 referenced normative values shown in Figure 2.4 are based on a body weight of 70 kg. The relationship between work rate and predicted $\dot{V}O_{2(ss)}$ is influenced by body weight according to the following equation:-

Predicted $\dot{V}O_2 = 5.8 \times$ Body weight (kg) + 151 $+10.1 \times$ Work Rate (W).....(19)
(Wasserman et al., 1987).

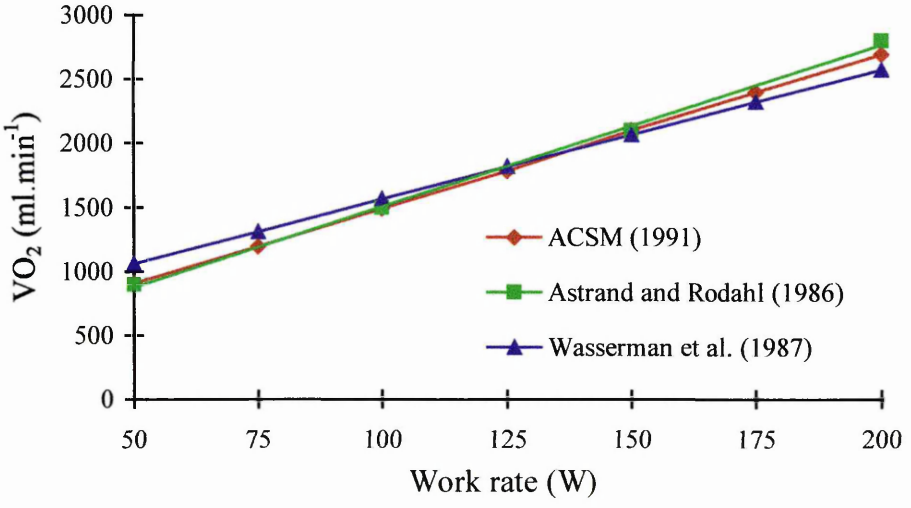


Figure 2.5. Oxygen Uptake at Increasing Work Rates (published data).

The gradients of the 3 oxygen uptake / work rate curves, shown in Figure 2.5, range between 10.1 $ml \cdot min^{-1}$ for the Wasserman et al. (1987) estimation, to 12.0 and 12.6

$ml \cdot min^{-1}$ for the ACSM (1991) and the Åstrand and Rodahl (1986) estimations respectively. This relates to an oxygen cost difference between the referenced sources of 165 $ml.min^{-1}$ at 50 watts, 35 $ml.min^{-1}$ at 150 watts and 223 $ml.min^{-1}$ at 200 watts. These differences are likely to result from a number of variables including methodology and the natural variation inherent in biological systems including differences in mechanical efficiency.

There is debate with regards to the inter-individual variability in the measure of mechanical efficiency, Åstrand and Rohdahl (1986) report mechanical efficiency to range from 19.6 to 28.8 % for cycling at pedal frequencies between 40 and 100 $rev \cdot min^{-1}$. Wasserman and Whipp (1975) consider the average mechanical efficiency to be approximately 30%. At 200 W the difference between the normative values shown in Figure 2.5 is less than 8% which may indicate that mechanical efficiency differences may account for most if not all of the differences in the reported data. Other factors such as saddle height (Hamley and Thomas, 1967) and crank length however have also been reported to influence $\dot{V}O_{2(ss)}$ (Carmichael et al., 1982).

It is essential that all tests should be carried out under standardised conditions, ideally the ambient temperature should be within 18°C and 22°C, with relative humidity no greater than 60% (British Association of Sports Sciences, 1988). The calibrant must be habituated to the exercise task and the test carried out at the same time of day to account for diurnal variation (Reilly, 1987). Unfamiliarity with test procedures may cause anxiety which is intrusive during sub-maximal tests (British Association of Sports Sciences, 1988). Finally, the calibrant should maintain a normal diet and not have been exercising prior to the test.

Although subject to a degree of variability, if a sound methodological approach is adopted and procedures standardised, the use of a physiological calibrant should provide important information about the performance of any gas analysis system.

2.4. The Measurement of Oxygen Uptake Kinetics

After the selection of appropriate technology which incorporates accurate data

collection at the desired resolution to measure $\dot{V}O_{2\,KINETICS}$, two further considerations must be addressed. Firstly, the selection of the method by which to describe the response and secondly selection of an appropriate experimental protocol in order to challenge the system.

2.4.1. Modelling the Oxygen Uptake Response

The simplest approach to describing the $\dot{V}O_2$ response to a transient work rate is to use a response time. This is the time taken for the $\dot{V}O_2$ to reach a predetermined level. This approach may be appropriate if the stimulus is short and there is a clearly definable peak to the response. More often a modification of this method, know as the half-time (t½), is preferred. Oxygen uptake t½ simply represents the time taken to reach one half of the final value. The method proposed by Auchincloss et al. (1966) is to determine t½ graphically by plotting the time course of the difference between steady state (Phase III) and the oxygen uptake response during the first 3 minutes of exercise on semi-logarithmic paper. The advantage of this method is that it can be applied directly to a range of input stimuli without the need for fitting complex models. However, to more fully understand the underlying system and its individual components, an appropriate mathematical model is required. The first example of this was the exponential model proposed by Henry (1951) whereby the data collected in response to a moderate step increase in work rate, was fit by the following equation using non-linear regression :

$$\dot{V}O_2(t) = \dot{V}O_{2\,(SS)}\,(1 - e^{-kt}) \quad \text{.....(20)}$$

where $\dot{V}O_2(t)$ is oxygen uptake at time t after the onset of exercise, $\dot{V}O_{2(SS)}$ is the amplitude of the Phase III steady state and e is the base of natural logarithms, t is the time in minutes, and k is the parameter of the curve (with the dimension of time^{-1}). Half-time $\dot{V}O_2$ can be calculated as :

$$t½ = -0.693/k \quad \text{.....(21)}$$

The convention for fitting the exponential model was modified to :

$$\dot{V}O_2(t) = \Delta\dot{V}O_{2(SS)}\,(1 - e^{-t/\tau}) \quad \text{.....(22)}$$

where $\Delta\dot{V}O_2$ is the change in $\dot{V}O_2$ above base line and τ is the time constant of the

exponential or the time to reach ≅63% [(1-e^{-1})x100] of the $\Delta\dot{V}O_{2(SS)}$ (Cooper et al., 1985) and is generally considered as a parameter of $\dot{V}O_{2\ KINETICS}$ at the onset of exercise (Hamar, 1991).

The relationship between τ, k and t½ :

$$\tau = \frac{1}{k} = \frac{t½}{0.693} \quad \text{.....(23)}$$

Fitting this single exponential (Equation 22) to the whole response does not differentiate between the cardiodynamic Phase I and the metabolic Phase II components and, although models have been proposed (Hughson and Swanson, 1989 for example) which employ two exponential equations in series to account for Phase I and Phase II independently, many researches (Hughson and Morrisey, 1982; Whipp, 1987) consider that a single exponential model with an additional time delay (D) (Equation 24) used to describe Phase II alone, should be employed in investigations of cellular oxygen metabolism.

$$\dot{V}O_2(t) = \Delta\dot{V}O_{2(SS)} \times (1 - e^{[-(t-D)/\tau]}) \quad \text{.....(24)}$$

The justification for removing the Phase I component from the model fit (often by omitting the first 15-20 s of data) is that Phase I does not reflect the $\dot{V}O_2$ response to the exercise task, but relates rather to the increased pulmonary blood flow, increased ventilation (Hamar, 1991) and the replenishment of the oxygen stores in blood pooled in non-working stores prior to exercise which are returned to the pulmonary circulation at the start of exercise (Krogh and Lindhard, 1913).

The use of a single exponential with time delay introduces an additional problem. Normally a computer program using an iterative process (non-linear regression - which minimises the square of the difference between the model and the data) is used to calculate the constants τ and D. It is argued that the best possible value for the time constant (τ) of the response is achieved without artificially constraining the regression to pass through the origin. This leads to difficulties in interpretation as this method generates both negative and positive time delays. To account for this Linnarson (1974) used the mean response time (MRT), the sum of D + τ to describe the response. The

MRT represents the time until the "centre of gravity" for a change has taken place, and is synonymous with the mean transit time used in connection with dye dilution studies of blood flow. Although MRT is considered a useful estimate of an overall rate of change of a response, a change in MRT will occur either as a result of a change in τ and/or a change in D. The calculation of MRT therefore obscures some of the fundamental characteristics of the response. Inman et al., (1987) applied this technique and recorded a mean rest to 100 W cycling MRT of ~25 s in six fit male subjects.

2.4.2. System Linearity

An important tool for the investigation of control mechanisms of physiological systems is the study of system linearity (Hughson, 1990).

The term linearity when applied to the oxygen uptake response has two important components a) steady state linearity and b) dynamic linearity.

2.4.2.1. Steady State Linearity

Steady state linearity can be demonstrated by considering the fact that for each W of power required during constant frequency pedalling on a cycle ergometer, the oxygen requirement is typically ~10 $ml \cdot min^{-1}$. That is to say $\dot{V}O_2$ is directly proportional to external work done in low-moderate intensity exercise. It is important to recognise that this relationship only holds true if the work rate input is of sufficient duration and low enough intensity to elicit Phase III steady state. High intensity exercise results in anaerobiosis and the accumulation of lactic acid, the oxidation of which alters the $\dot{V}O_2$ / work rate relationship (Paterson and Whipp, 1991; Whipp and Mahler, 1980) and the system no longer exhibits steady state linearity.

2.4.2.2. Dynamic Linearity

Dynamic linearity implies that the time constant (τ) of the $\dot{V}O_2$ response is largely independent of work rate, prior conditions, and of the type of work rate forcing function (Hughson, 1990). Take for example the $\dot{V}O_2$ response to a step change in work rate as described above. The response is not instantaneous but is controlled by mechanisms associated with either the transport of oxygen to the working muscles, or with the

process of cellular respiration itself. Under normal conditions if these mechanisms do not change in characteristic then the rate of the $\dot{V}O_2$ response will not be determined by extraneous factors such as the intensity of exercise, previous conditions or the way in which the work rate increase is applied, but by factors inherent in the response itself. That is to say the rate of increase in cellular oxygen uptake to a change in work intensity is relatively fixed, under normal conditions.

2.4.2.3. Principle of Superposition

If the $\dot{V}O_2$ response demonstrates both dynamic and steady state linearity then the principle of superposition will apply. This means that the response should be predictable in terms of time course and amplitude (Hughson, 1990) and theoretically, as long as the linearity of the power-$\dot{V}O_2$ relationship is not severely violated (Hoffmann et al., 1994a) the characteristics of the response will be unaltered even when subjected to a range of different work rate stimuli. In physiologic systems where the principle of superposition applies, there is potential for studying the physiologic responses to absolute intensity work rate protocols without the necessity to establish relative work intensities. This is important when comparing subjects of different fitness levels or body size.

2.4.3. Experimental Protocols

A range of different work rate perturbations have been used in the evaluation of $\dot{V}O_{2\,KINETICS}$ each of which require different methods of analysis. The five most frequently employed exercise transitions are the step (square), impulse, ramp, sinusoid and the PRBS work rate perturbations. The responses to these work rate stimuli may be analysed in two fundamentally different ways. The traditional approach of fitting a model to the $\dot{V}O_2$ response plotted against time to determine τ, D and MRT is considered appropriate for analysis of the step, impulse, and ramp perturbations, because fairly simple mathematical models can be constructed to fit the response. The response to the PRBS is somewhat more complex and analysis of this signal is achieved either in the time domain after cross correlation (Hughson et al., 1991b) or in the

frequency domain (Hughson et al., 1991c). Although impulse like "burst" and ramp protocols have been used in the measurement of $\dot{V}O_{2\,KINETICS}$ in children (Zanconato et al., 1991 and Cooper et al., 1984 respectively) there are problems with the interpretation of these results. For example, in the low intensity, 1-minute "burst" exercise performed by Zanconato et al. (1991), the noise to signal ratio was too high and the test duration too short, to allow accurate data analysis. Although, it has been reported (Whipp et al. 1981; Davis et al. 1982) that, for appropriate ramp increases in work rate, valid estimates of τ can be determined, more recently, the experimental evidence of Hughson and Inman (1986a) and Swanson and Hughson (1988) indicates that estimates of $\dot{V}O_{2\,KINETIC}$ parameters using the single ramp test (for example Cooper et al. 1984) should be interpreted with caution due to marked variability between tests. Therefore, due to the apparent simplicity of the step transition in work rate, it has become the preferred method of measuring Phase II $\dot{V}O_{2\,KINETICS}$ (Whipp, 1971; Diamond et al., 1977; Whipp et al., 1982; Cooper et al., 1985; Whipp 1987; Di Prampero et al., 1989; Hoffmann et al., 1994a).

2.4.3.1. Step Transitions in Work Rate

Step transitions either from rest or from low intensity to moderate intensity exercise offer an obvious method of measuring $\dot{V}O_{2\,KINETICS}$. The procedure lasts 8 –12 minutes and ideally is repeated several times and the resultant $\dot{V}O_2$ responses from the repetitions averaged in order to minimise the effects of breath-to-breath variability (Lamarra et al., 1987) on the signal response. The confidence with which the kinetic parameters of τ and D can be estimated is dependent upon the size of the breath-to-breath variability in the signal. Lamarra et al. (1987) proposed that in situations where the breath-to-breath variation can be characterised as Gaussian and uncorrelated then the number of repetitions n required for a desired confidence interval K_n is given by :-

$$n = \left[\frac{\hat{L} \cdot s_b}{K_n \cdot \Delta Yss} \right]^2 \quad \text{.....(25)}$$

where $\hat{L}$ is a constant and s_b is the standard deviation of the baseline response and ΔYss is the difference in the steady-state values of oxygen uptake between baseline and

moderate intensity exercise.

In moderate intensity exercise, Phase II $\tau\dot{V}O_2$ is not appreciably influenced by the step amplitude (Whipp, 1971; Diamond et al., 1977; Whipp, 1987; Hoffman et al., 1994a). This means that the early transient rise in blood lactate (Cerretelli and Di Prampero, 1987) that is not uncommon at these work rates does not seem to influence the response discernibly (Whipp, 1994). There is however some controversy surrounding the issues relating to the dynamic linearity of the step response, for example, faster $\dot{V}O_{2\,KINETICS}$ have been reported when the step transition is made from rest compared to work to work transitions (Hagberg et al., 1978; Hughson and Morrissey, 1982; DiPrampero et al., 1989). However, the different strategies for modelling the $\dot{V}O_2$ response can markedly influence the interpretation. Whipp et al., (1982) considered three models for characterising the kinetic behaviour of $\dot{V}O_2$ all of which were based upon the exponential model described in Equation 24 (Section 2.4.1). The first approach constrained the model to start at the onset of exercise (Time delay = 0); model 2 incorporated a time delay and, in model 3, the response was constrained to start only at the end of Phase I. It was concluded that the most appropriate model for estimating $\tau\dot{V}O_2$ is when the $\dot{V}O_2$ change during Phase I is excluded from the model fit. The strategy adopted by both Hagberg et al. (1978) and DiPrampero et al. (1989) in order to investigate the response time to a step increase in work rate was to calculate $t½\ \dot{V}O_2$. The analysis was therefore inappropriate to interpret the Phase II response.

The concept of the step change as a protocol for investigating $\dot{V}O_{2\,KINETICS}$ may appear to be remarkably simple but the practicalities involved in removing noise by superimposing several repetitions (Whipp et al., 1982; Hughson et al., 1988; Lamarra et al., 1987) and difficulties associated with both the analysis and interpretation of the results after fitting explicit mathematical models make the technique a challenging one. Although the mathematical models are reasonably well defined they can be to some degree arbitrary as it is still a matter of debate as to what extent the various possible mechanisms contribute to total $\dot{V}O_2$ (Eßfeld et al., 1987). An alternative approach which obviates the need to select an appropriate model is to analyse the response in the

frequency domain i.e. the sinusoidal exercise perturbation (Casaburi et al., 1977; Cunningham et al., 1993; Haouzi et al., 1993; Hoffman et al., 1992 and 1994b) and the multi-frequent PRBS test (Eßfeld et al., 1987; Hughson et al., 1991b; Kusenbach et al., 1994).

2.4.3.2. Sinusoidal Transitions in Work Rate

The sinusoidal work rate transition is a continuous signal which is characterised by how often it oscillates about a predetermined intensity (frequency) and the size of these oscillations (amplitude). The frequency (measured in Hz) is a measure of how often the sinusoid repeats each second and is calculated as the inverse of the period. The period of the sinusoidal oscillation is the time (measured in seconds) to complete a full cycle. The amplitude of the cycle ergometer work rate transition is measured in Watts.

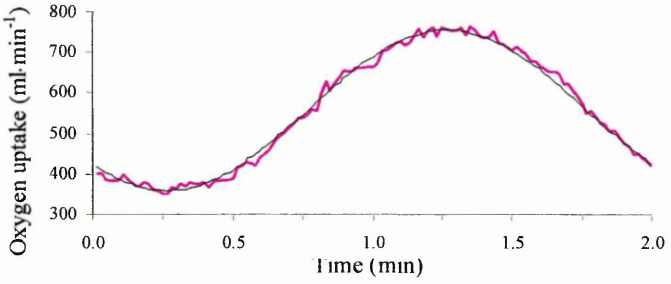


Figure 2.6. The oxygen uptake response to a 16-min sinusoidal work rate forcing with a period of 2-min. The work rate oscillated between 25 and 85 W and the data shown are the average of the last six complete cycles.(Based on Casaburi et al. 1977)

The $\dot{V}O_2$ response ($ml \cdot min^{-1}$) to a sinusoidal exercise stimulus shown in Figure 2.6 has been observed to be linear and first order to a range of sinusoidal frequencies below the lactate threshold (Casaburi et al., 1977; Hoffmann et al., 1994a). That is, the characteristic $\dot{V}O_2$ response to the sinusoidal work rate input will be a sine wave of the same frequency but of a different amplitude and, as the frequency of input work rate is increased, the $\dot{V}O_2$ response has a reduced amplitude and a greater delay (Casaburi et al., 1977; Hughson, 1990) Figure 2.7.

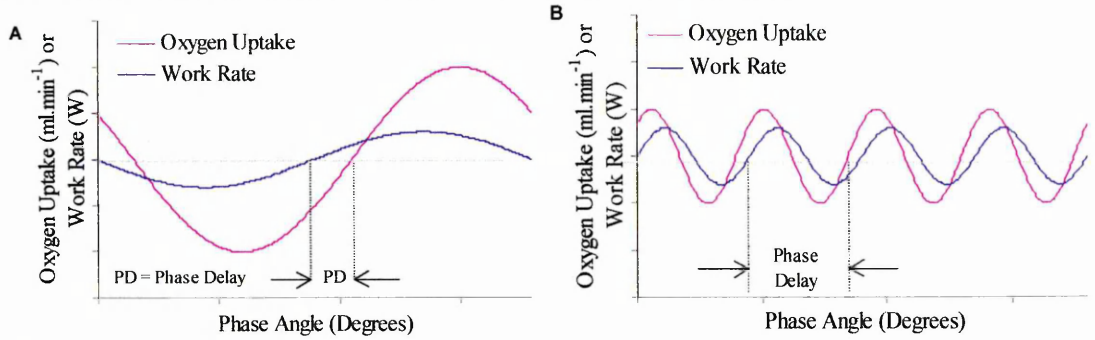


Figure 2.7. Schematic of the $\dot{V}O_2$ response to a low frequency (A) and a high frequency (B) sinusoidal work rate protocol. Note the larger delay in the response at the higher frequency.

The results of a range of sinusoidal frequencies tested by Casaburi et al. (1977) showed that the phase delay increased from a range of 18 to 34 degrees to a range of 51 to 91 degrees, and the amplitude of the response fell from a range of 170 to 470 ml·min^{-1} to a range of 23 to 101 ml·min^{-1} over the increasing frequency range of 1.67 to 23.81 mHz.

It has been suggested that for lower frequency inputs, for example those corresponding to frequencies of 2.2 to 13.3 mHz (Hughson et al., 1990a), it is the second component of the $\dot{V}O_2$ dynamic response (Phase II) that dominates. The work of Hoffmann et al. (1994b) also concluded that in the lower frequency range, the $\dot{V}O_2$ response to sinusoidal work rate transitions shows only little, if any, influence of the circulatory and pulmonary system (Phase I). Hoffmann et al. (1994b) report that the $\dot{V}O_2$ response to sinusoidal transitions, within clearly defined conditions, meets the criteria for both static and dynamic linearity. These conditions are that the maximal workload applied should be below the anaerobic threshold for all subjects as significant changes in lactate are associated with alterations in the work rate / $\dot{V}O_2$ relationship. Also, there should be no rest to exercise transitions as these type of transitions can induce the fast cardiovascular (Phase I) adjustments. The final recommendation is that the period of the input signal should be in excess of 100s as responses to the higher frequency range are distorted by non-linear physiological mechanisms and / or biological (breath-to-breath variation) or technical noise.

The application of moderate intensity sinusoidal work rate perturbations to exercise testing has reduced the need for modelling the $\dot{V}O_2$ response, thus providing a more

direct means of measuring $\dot{V}O_{2\,KINETICS}$ and theoretically providing a more accurate and reliable method than that achieved by using a step (Hamar, 1991).

The sinusoidal approach has the limitation that only one frequency can be assessed at a time. The advantage of the PRBS test is that the signal is used to measure the frequency response at a number of harmonic frequencies simultaneously.

2.4.3.3. Pseudo-random Binary Sequence Work Rate Transitions

The PRBS exercise protocol consists of a number of units, the length of the unit represents the minimum duration of time maintained at either of two intensities of work. It is the duration of the unit which dictates the harmonic frequencies to be stimulated. The number of units making up the protocol is dictated by an algorithm :

$$\text{Units} = 2^n - 1 \quad (26)$$

For example, when n = 4 the number of units in the PRBS protocol is 15 and when n = 6 the number of units in the sequence is 63. The majority of the studies published have been based on PRBS sequences with either 63 units of 5 s duration or 15 units of 30 s duration (Bennett et al., 1981; Eßfeld et al., 1987, respectively). The choice of these signals was based on the duration of each complete sequence being 300 and 450 s respectively, thereby permitting several sequences to be delivered consecutively whilst still restricting the overall test duration to around 30 minutes.

Based on results of studies using a 15 units of 30 s duration, PRBS protocol, Eßfeld et al. (1987) and Stegemann et al. (1985) have concluded that any alteration in the amplitude or phase delay response in this relatively low frequency range must be a consequence of non-haemodynamic factors i.e. Phase II. Only when the frequency content of the input signal is high enough, for example, in a 63 units of 5 s duration PRBS, does the rapid initial component become apparent in the Fourier analysis (Hughson et al., 1990a). Although the 15 unit of 30s duration PRBS does excite the faster components it puts greater emphasis on the more slowly adapting Phase II responses (Hughson et al., 1991b). The PRBS protocol itself (Figure 2.8) is generated with a digital shift register with modulo-2 adder feedback (Kerlin, 1974). The method of

constructing a 15 unit PRBS protocol is described in full in Appendix 2.1.

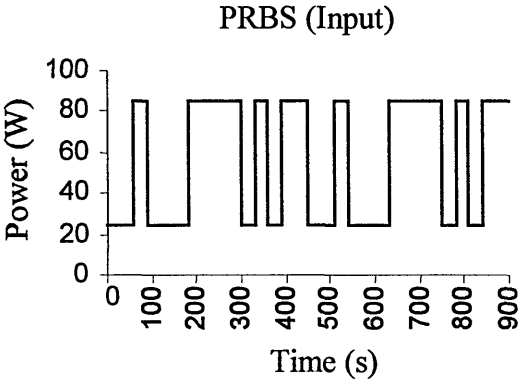


Figure 2.8. Two, consecutive pseudo-random binary sequence exercise protocols consisting of 15 units of 30 s duration.

The PRBS exercise tests provide a description of $\dot{V}O_{2\,KINETICS}$ for a range of input sinusoidal frequencies in a single sub-maximal assessment (Hoffmann et al., 1994b). The rationale behind the PRBS exercise test relies on the principle of superposition. That is, in a linear system, the response to the PRBS signal may be considered as a sum of its individual harmonic (sinusoidal) components. Using Fourier analysis (Appendix 2.2) the $\dot{V}O_2$ response is transformed from the time domain into the frequency domain, essentially breaking the response down into its constituent harmonic components. The relationship between the ergometer input and $\dot{V}O_2$ output is then expressed as an amplitude ratio ($ml \cdot min^{-1} \cdot W^{-1}$) and a phase delay (degrees) as follows :-

$$\text{Amplitude ratio } (ml \cdot min^{-1} \cdot W^{-1}) = \frac{\dot{V}O_2 \text{ Amplitude}}{\text{Work Rate Amplitude}} \quad \text{.....(27)}$$

$$\text{Phase delay (degrees)} = \text{Work rate phase angle} - \dot{V}O_2 \text{ phase angle} \text{.....(28)}$$

An infinite number of harmonics can be derived in this manner, but after the work of Hoffman et al. (1994b) only those parameters in the frequency range 2.2- 8.9 mHz were considered suitable for analysis.

The pattern of response of the harmonic components is identical to the response that would have been obtained if the individual sinusoidal work rate perturbation had been

applied. That is, as the frequency of the work rate change is shortened, the $\dot{V}O_2$ output has a reduced amplitude and a greater phase delay (c.f. Figure 2.7).

In an investigation to examine specifically the dynamic linearity of the PRBS protocol, Hughson et al. (1991b) demonstrated that the time constants of the $\dot{V}O_2$ dynamic response, estimated from step and PRBS, were not significantly different. They concluded that the indicators of system dynamics, measured using the PRBS protocol, are similar to those investigated during step changes in work rate. The PRBS has also been validated as a test of $\dot{V}O_{2\,KINETICS}$ by comparing the responses with those of individual sine wave responses (Hoffman et al., 1992 and 1994b). Provided the frequency responses are measured for periods greater than 100s the $\dot{V}O_{2\,KINETICS}$ response has been shown to be both statically and dynamically linear. The PRBS technique is therefore said to provide a superior input signal for the assessment of $\dot{V}O_2$ transients (Eßfeld et al., 1982) and has been shown to be an appropriate method of assessing the $\dot{V}O_{2\ KINETICS}$ of children in response to exercise below the lactate threshold (Kusenbach et al., 1994).

2.5. Factors Controlling Phase II Oxygen Uptake Kinetics

Phase II of the $\dot{V}O_2$ response to a step change in exercise intensity is described by first order exponential kinetics suggesting that there is a single or pre-dominant rate limiting step for $\dot{V}O_{2\,KINETICS}$. There are two theories concerning where this rate limiting step lies:

- in the oxygen delivery to the working muscle (central limitation)
- in the ability of the muscle to utilise oxygen (peripheral limitation).

Two approaches have been used to provide support for these theories. One is to alter one or more steps in the oxygen delivery process, and the other is to identify the physiological variable with the same response characteristics as the $\dot{V}O_2$ response.

2.5.1. Acute Alterations of the Oxygen Delivery Process

This approach involves changing the rate of oxygen delivery to the working muscle through interventions involving β-adrenergic receptor blockade, hypoxia and hyperoxia,

circulatory occlusion, upright and supine exercise, and finally techniques which create negative and positive lower body pressures. If oxygen transport is limiting then any change in oxygen transport should be directly linked to changes in $\dot{V}O_{2\,KINETICS}$.

2.5.1.1. β-adrenergic Receptor Blockade

Drugs which block β-adrenergic receptors inhibit the effects of sympathetic nervous stimulation. Cardio-selective drugs have their main effect on the β_1-adrenergic receptors in the heart, whereas non-selective β-adrenergic receptor blocking drugs have a more general effect on β_2-adrenergic receptors throughout the body (Yeo et al., 1991). The effects that non-selective β-adrenergic receptor blocking drugs have on the exercise response include a reduction in cardiac output, decreased fat and carbohydrate mobilisation and an impairment of the normal redistribution of blood flow that occurs during exercise (Yeo et al., 1991). It might be anticipated that $\dot{V}O_{2\,KINETICS}$ should be slower due to the lower cardiac output and poorer blood flow redistribution with β-adrenergic receptor blockade. This has been shown in various studies (Hughson and Smyth, 1983; Hughson, 1984; Kowalchuk and Hughson, 1990). It made no difference whether the drug administered was a cardio-selective or a non-selective β-adrenergic receptor blocking agent. Although this suggests a central limitation for $\dot{V}O_{2\,KINETICS}$ these studies have been criticised because the reduced exercise capacity of healthy people receiving β-adrenergic receptor blocking agents may have resulted in the subjects exercising at an upper work rate which was no longer truly aerobic (Hoffmann et al., 1994a). This may explain the slower $\dot{V}O_{2\,KINETICS}$ during β-adrenergic receptor blockade.

2.5.1.2. Hypoxia and Hyperoxia

Hypoxia has been shown to cause a significant slowing in the $\dot{V}O_2$ response during ramp, step and PRBS work rate protocols (Murphy et al., 1989; Hughson and Kowalchuk, 1995) when compared to normoxia. This suggests that oxygen transport is a rate limiting step in oxidative metabolism. It therefore follows that if oxygen transport is limiting, then hyperoxia should result in faster $\dot{V}O_{2\,KINETICS}$. Administering gas

mixtures containing 70% oxygen did not affect Phase II $\dot{V}O_{2\,KINETICS}$ during exercise (Hughson and Kowalchuk, 1995) suggesting that oxygen transport can act as a rate limiting step if inspired oxygen is reduced, but under conditions of normal arterial oxygen content, oxygen transport does not become limiting. In a study using inspired gas mixtures containing 40% oxygen (Kusenbach et al., 1999), enhanced amplitude ratios were demonstrated in healthy control subjects in response to a 15 unit of 30 s duration PRBS protocol. This might indicate a cardiopulmonary limitation to $\dot{V}O_{2\,KINETICS}$ in their population that was not apparent in the subjects of Hughson and Kowalchuk (1995).

2.5.1.3. Circulatory Occlusion

Circulatory occlusion of both legs, after elevation to improve venous drainage, increases central blood volume. This manoeuvre should temporarily increase cardiac output, and has been shown to speed up $\dot{V}O_{2\,KINETICS}$ during arm exercise (Hughson and Inman, 1986b). It is therefore suggested that $\dot{V}O_{2\,KINETICS}$ during arm exercise may be limited by arm blood flow.

2.5.1.4. Supine and Upright Exercise

Variations in body position bring about circulatory changes which may have opposing effects on muscle perfusion. In the supine position, venous return and therefore cardiac output, increase due to the removal of the normal gravitational effects seen in upright exercise. Even though cardiac output is increased during supine exercise (Hughson et al., 1991c) it may not be reflected by an increased muscle perfusion for two reasons. Firstly, during supine exercise there may be a decrease in sympathetic tone to the splanchnic beds that would cause a reduction in the normal vasoconstriction seen during exercise. When compared to upright exercise, less blood would flow to the active skeletal muscle during supine exercise as more blood flows to the splanchnic area. Secondly, the decreased arterial pressure during supine exercise may decrease the perfusion pressure across the muscle capillary beds and consequently decrease muscle blood flow. This theoretical decrease in muscle perfusion may explain the slower

$\dot{V}O_{2\,KINETICS}$, in response to a step change in work rate, observed during supine exercise when compared to upright exercise (Hughson et al., 1990b, and Convertino et al., 1984). Concurrent measurement of $\dot{V}O_2$ and leg blood flow during knee extension exercise have shown that $\dot{V}O_{2\,KINETICS}$ and blood flow kinetics were both slowed in the supine position compared with the upright position (MacDonald et al., 1998). No evidence was found in this study that leg blood flow limited $\dot{V}O_{2\,KINETICS}$ in upright exercise.

Other studies, using the PRBS exercise test, have failed to demonstrate a significant slowing of $\dot{V}O_{2\,KINETICS}$ in the supine position (Hoffmann et al., 1991 and 1994a). Hughson et al. (1991c) showed a trend towards slower $\dot{V}O_{2\,KINETICS}$ in the supine position but significant differences were only found at the frequency of 6.7 mHz.

In summary, supine and upright experiments have produced conflicting results which is not surprising considering the partially opposing circulatory effects brought about by changing body position. Measurement of muscle perfusion during upright and supine exercise may explain the role of oxygen transport in determining $\dot{V}O_{2\,KINETICS}$ during sub-maximal exercise.

2.5.1.5. Lower Body Negative and Positive Pressure

Application of lower body negative pressure (LBNP) has been used as a technique to increase leg muscle blood flow (Eiken, 1988; Hughson et al., 1993). Lower body negative pressure increases muscle blood flow by decreasing the pressure in the capacitance vessels, thereby increasing the arterio-venous oxygen pressure gradient across the muscle bed. In supine exercise, the application of LBNP increased $\dot{V}O_{2\,KINETICS}$ to values that were similar to those seen in upright exercise. This would suggest that the decrease in $\dot{V}O_{2\,KINETICS}$ seen in supine exercise is due to a decrease in oxygen delivery to the muscle, since it can be reversed by a technique which increases oxygen delivery. Evidence from LBNP studies supports the central limitation theory for $\dot{V}O_{2\,KINETICS}$.

Conversely, lower body positive pressure (LBPP) decreases muscle blood flow (Sundberg and Kaisjer, 1992) and it would be expected that application of LBPP would

decrease $\dot{V}O_{2\,KINETICS}$. The application of a LBPP of 45 torr, however, failed to alter $\dot{V}O_{2\,KINETICS}$ (Williamson et al., 1996) casting doubt on the suggestion that $\dot{V}O_{2\,KINETICS}$ under normal conditions are limited by blood flow to the working muscle.

2.5.1.6. Prior Activity

In an assessment of the effects of both sub- and supra- lactate threshold (LT) warm up on $\dot{V}O_{2\,KINETICS}$, Gerbino et al. (1996) reported that neither prior sub- nor supra-LT exercise effects the $\dot{V}O_{2\,KINETICS}$ of subsequent sub-LT exercise. However, in conditions of supra-LT exercise the calculated "effective" $\dot{V}O_2$ time constant was shortened by an amount in the order of 30% in response to prior supra-LT exercise. Sub-LT warm up had no such influence on subsequent supra-LT exercise. Although the mechanisms responsible for these observations have not been firmly established it was suggested that the residual metabolic acidemia from the supra-LT warm up bout leads to an improved muscle perfusion during exercise which consequently speeded the supra-LT $\dot{V}O_{2\,KINETICS}$.

2.5.1.7. Acute Alterations of the Oxygen Delivery Process - Summary

Although some manipulations which decrease oxygen delivery to the muscle have been shown to decrease $\dot{V}O_{2\,KINETICS}$, the lack of a consistent effect of increasing oxygen delivery on $\dot{V}O_{2\,KINETICS}$ suggests that oxygen delivery may not limit $\dot{V}O_{2\,KINETICS}$ under normal conditions.

2.5.2. Identification of the Physiological Variable with the Same Response Characteristics as the Oxygen Uptake Response

2.5.2.1. Heart Rate and Cardiac Output Kinetics

If the rate of oxygen delivery to the muscle is the limiting factor for $\dot{V}O_{2\,KINETICS}$ then the rate of response of the oxygen delivery system should be similar to the $\dot{V}O_2$ response. The rate of increase in $\dot{V}O_2$ is often found to be slower than the rate of increase in either heart rate or cardiac output at the onset of exercise (Cerretelli et al., 1966; Linnarsson, 1974; De Cort et al., 1991; Yoshida and Whipp, 1994). Although these studies might suggest that cardiac function does not limit $\dot{V}O_{2\,KINETICS}$, the effect

of redistribution of blood flow within the muscle is not known and therefore these studies do not preclude the possibility of oxygen delivery limiting $\dot{V}O_{2\,KINETICS}$.

Hughson and Morrisey (1983) used the approach of comparing the change in heart rate kinetics with changes in $\dot{V}O_{2\,KINETICS}$, using a range of different step exercise transitions. The similar changes in both $\dot{V}O_2$ and heart rate kinetics were taken as evidence of a central limitation for $\dot{V}O_{2\,KINETICS}$ assuming that the heart rate kinetics reflect muscle blood flow kinetics.

2.5.2.2. Blood Flow Kinetics

Muscle blood flow increases very rapidly at the beginning of exercise (Walloe and Wesche, 1988; Eriksen et al., 1990; Grassi et al., 1996). In order to establish the role of muscle blood flow and, therefore oxygen delivery as a limiting factor for $\dot{V}O_{2\,KINETICS}$, the $\dot{V}O_{2\,KINETICS}$ of the working muscle have been measured simultaneously with muscle blood flow (Hughson et al., 1996; Grassi et al., 1996). Grassi et al. (1996) measured the $\dot{V}O_{2\,KINETICS}$ of the leg, by frequent measurement of the $C(a-\bar{v})O_2$ across the leg, and $\dot{V}O_{2\,KINETICS}$ at the mouth during leg cycling exercise at 50 W. As $\dot{V}O_{2\,KINETICS}$ measured at the mouth and leg blood flow had the same time constants, it might be concluded that blood flow was limiting $\dot{V}O_{2\,KINETICS}$. As the time delay between an increase in muscle $\dot{V}O_2$ reflects an increase in pulmonary $\dot{V}O_2$, it is important to compare $\dot{V}O2$ at the muscle with leg blood flow. This comparison shows that leg muscle $\dot{V}O2$ increased only modestly in the first 10 to 15 seconds of exercise even though blood flow increased markedly. This suggests that, early in the exercise response, bulk delivery of oxygen to the working muscle is not limiting $\dot{V}O_{2\,KINETICS}$ of the leg, but it is still not possible to discriminate between maldistribution of blood flow within muscle and the effects of peripheral mechanisms controlling the response. At present, techniques are not available to measure the complex blood flow patterns in exercising muscle in humans.

There is some evidence that blood flow limits $\dot{V}O_{2\,KINETICS}$ in the arms when blood flow is reduced by carrying out arm exercise above the level of the heart (Hughson et al.,

1996). Studies using leg exercise have also shown that reduced blood flow is associated with reduced $\dot{V}O_{2\,KINETICS}$ in the supine position (MacDonald et al, 1998). Until experiments are performed to investigate the simultaneous effect on $\dot{V}O_{2\,KINETICS}$ of increasing blood flow above normal during exercise, the role of blood flow limitation on $\dot{V}O_{2\,KINETICS}$ will remain controversial.

2.5.2.3. Phosphocreatine Kinetics

Another technique, using the same approach, is to compare $\dot{V}O_{2\,KINETICS}$ with the kinetics of phosphocreatine (PC) degradation. It has been proposed that $\dot{V}O_{2\,KINETICS}$ of the muscle and therefore $\dot{V}O_{2\,KINETICS}$ measured at the lung, reflect the metabolic processes that control mitochondrial respiration in the exercising muscle (Mahler, 1985; Meyer, 1988; Whipp and Ward, 1990). The rate of PC degradation has been implicated as the rate limiting step in the control of mitochondrial respiration (Connett and Honig, 1989). Studies which have measured $\dot{V}O_{2\,KINETICS}$ and PC kinetics have therefore provided some insight into the role of peripheral mechanisms in limiting $\dot{V}O_{2\,KINETICS}$. Studies on animals muscle preparations (Piiper and Spiller, 1970; Mahler, 1985) have reported that the time course of the change in [PC], during recovery from a period of artificially induced tetanus, is directly proportional to the time course of $\dot{Q}O_2$ in well oxygenated muscle. In intact animals Marconi et al. (1982) has also demonstrated that, in well oxygenated muscle, the time constants for $\dot{Q}O_2$ and $\dot{V}O_2$ are similar. In humans PC breakdown has been estimated by both nuclear magnetic resonance imaging (^{31}P-NMR) and muscle biopsy techniques, both in isolated muscle preparations and *in situ* during voluntary muscle contractions (Coggan et al., 1993). Barstow et al. (1994) measured the time constants for Phase II $\dot{V}O_{2\,KINETICS}$ and PC kinetics during two different exercise modes which provided indirect evidence of this link since the time constants for Phase II $\dot{V}O_{2\,KINETICS}$ were similar to those for PC degradation. In a more recent study, McCreary et al. (1996) measured $\dot{V}O_{2\,KINETICS}$ and PC kinetics during plantar flexion and found time constants of 44.5 s for $\dot{V}O_2$ and 47 s for PC. The similarity between these time constants again supports the contention that $\dot{V}O_{2\,KINETICS}$

are controlled by muscle oxidative function with PC degradation controlling mitochondrial oxidative phosphorylation by the "creatine phosphate shuttle".

The $\dot{V}O_2$ of the skeletal muscle during exercise is both preceded by, and controlled by, the energy transferring reactions of the intramuscular high energy pool. Although the particular details of the controlling mechanisms remain controversial, mitochondrial creatine kinase appears to play a pivotal role, since the proportional exponential increase in muscle $\dot{V}O_2$ parallels the decrease in the levels of PC. Other controlling factors may be the phosphorylation potential [ADPx(Pi/ATP)] or the free energy for ATP splitting (Meyer and Foley, 1996)

The model presented by Mahler (1985) suggests that $\dot{V}O_{2\,KINETICS}$ are controlled by the availability of creatine (C), which in turn, is determined by how quickly PC degradation occurs at the myofibril. The activity of the mitochondrial creatine kinase enzyme is controlled by the phosphocreatine to creatine ratio (PC/C). In this model, describing the control of mitochondrial respiration during moderate exercise below the lactate threshold, oxygen is not thought to be limiting until the partial pressure of oxygen within the mitochondria (mitochondrial PO_2) falls below 1 mmHg. The oxygen supply to the mitochondria may become limiting when the partial pressure of oxygen in the capillary (capillary PO_2) falls to 15 to 20 mmHg (Wittenberg and Wittenberg, 1989) because of the physical factors limiting oxygen diffusion. In moderate exercise the partial pressure of oxygen in the veins (venous PO_2) remains above this level (Doll et al., 1968) suggesting that oxygen supply to the mitochondria does not become critical unless, as previously discussed, there is some maldistribution of the blood flow within the capillary bed.

2.5.2.4. Identification of the Physiological Parameter with the Same Response Characteristics as the Oxygen Uptake Response - Summary

Of the physiological variables studied, PC kinetics are most closely aligned to $\dot{V}O_{2\,KINETICS}$ and this, taken together with the current theories on control of mitochondrial respiration (Figure 2.3), provide some evidence for peripheral limitation of $\dot{V}O_{2\,KINETICS}$.

2.5.3. Conclusion

Whether the limiting factor determining Phase II $\dot{V}O_2$ kinetics is due to central or peripheral factors remains controversial. Theoretically it could be argued that the control of $\dot{V}O_{2\,KINETICS}$ is driven by ATP demand at the muscle (i.e. peripherally) rather than the supply of oxygen to the muscle. Until it becomes technically possible to measure PO_2 at the level of the muscle fibre in humans it seems likely that this controversy will remain.

2.6. Oxygen Uptake Kinetics: Applications

2.6.1. Clinical Applications

The measurement of $\dot{V}O_{2\,KINETICS}$ during exercise is a technique that has been applied clinically (Nery et al 1982; Sietsema, 1992; Koike et al., 1994; Casaburi et al., 1997; Kusenbach et al., 1999). The use of below lactate threshold exercise tests is preferable in severely ill patients because of fewer adverse events and the lower motivational requirement compared with that demanded by higher intensity tests. In the specific field of cardiac medicine the technique has been successfully applied to the assessment of the severity of cardiac disease (Koike et al., 1994). Similarly the technique has been used to evaluate rehabilitation programmes in patients suffering from chronic obstructive pulmonary disease (Casaburi et al., 1997).

2.6.2. Healthy Individuals

In healthy individuals, Phase II $\dot{V}O_{2\,KINETICS}$ have been shown to be affected by physical fitness (Powers et al., 1985; Eßfeld et al., 1987; Zhang et al., 1991; Chillibeck et al., 1996) endurance training (Hagberg et al., 1980, Berry and Moritani, 1985; Yoshida et al., 1992; Babcock et al., 1994a; Phillips et al., 1995; Norris and Petersen, 1998; Edwards et al., 1999) and by factors relating to ageing after the age of 29 years (Babcock et al., 1994b; and 26 years Chillibeck et al., 1996). Although a decline in $\dot{V}O_{2\,MAX}$ from the ages of 15 years to 71 years has been reported (Jones et al. 1985) the cross sectional studies which compare a group of children with a group of adults have provided conflicting evidence for children possessing faster $\dot{V}O_{2\,KINETICS}$ compared

with adults (Macek and Vavra, 1980a and b; Sady, 1981; Freedson et al., 1981; Sady et al., 1983; Macek et al., 1984; Cooper et al., 1984 and 1985; Armon et al., 1991; Springer et al., 1991; Zanconato et al., 1991 and Hebestreit et al., 1998).

2.6.2.1. Relationship between Maximum Oxygen Uptake and Oxygen Uptake Kinetics

Support for the positive relationship between $\dot{V}O_{2\,MAX}$ and $\dot{V}O_{2\,KINETICS}$ was derived from the work of Whipp and Wasserman, (1972), Hickson et al. (1978), Hagberg et al. (1978) and Powers et al (1985). These studies demonstrated that the $t½\dot{V}O_2$ was significantly shorter in subjects with higher aerobic power. The method of calculating $t½\dot{V}O_2$ in these investigations does not consider the possible effect of the cardio-dynamic influence (Phase I) on the speed of the $\dot{V}O_2$ response. Faster Phase II $\dot{V}O_{2\,KINETICS}$ have however been reported in individuals with high aerobic power compared to those with lower aerobic power in the studies of Powers et al. (1985) and Zhang et al. (1991). The more rapid $\dot{V}O_{2\,KINETICS}$ seen in the aerobically fit (higher $\dot{V}O_{2\,PEAK}$) subjects, were reported to indicate that the kinetics of aerobically produced ATP flux relative to the anaerobic ATP flux are relatively greater in the fitter subjects (Zhang et al., 1991). In a study by Chillibeck et al. (1996) investigating the effects of ageing on $\dot{V}O_{2\,KINETICS}$ it was concluded that although $\dot{V}O_{2\,KINETICS}$ are definitely slowed with age, relative levels of cardiorespiratory fitness also have a great influence on the dynamic response of $\dot{V}O_2$.

Eßfeld et al. (1987) made a comparative study of the relationship of $\dot{V}O_{2\,KINETICS}$ and aerobic capacity in 29 males and 9 female subjects (mean age 23.3 ± 3 years) using a 15 unit of 30 s duration, PRBS protocol. The sub-T_{vent} work rates used alternated between 20 and 80 W. The analysis technique incorporated a combination of auto- and cross-correlation prior to Fourier analysis in order to reduce the effect of irregular breathing patterns (noise). Subjects with higher $\dot{V}O_{2\,MAX}$ were found to achieve higher amplitude ratios (Table 2.2).

Eßfeld et al. (1987) has shown that it is possible to differentiate between subjects of different aerobic power by using a sub-maximal PRBS measurement of $\dot{V}O_{2\,KINETICS}$.

At the higher frequencies, differences in $\dot{V}O_2$ dynamics become apparent: the higher the relative $\dot{V}O_{2\,MAX}$, the higher the amplitude ratio at a given frequency.

Table 2.2. Amplitude ratio ($ml \cdot min^{-1} \cdot W^{-1}$) across frequency range 2.2 to 8.9 mHz for 29 males and 9 females grouped by aerobic power (derived from Eßfeld et al. 1987). Mean (S.D.)

	Frequency (mHz)			
$\dot{V}O_{2\,MAX}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.2	4.4	6.7	8.9
< 50	9.55 (1.15)	7.76 (1.15)	6.17 (1.66)	3.89 (1.74)
50 to 60	10.72 (1.15)	9.55 (1.15)	8.13 (1.23)	5.25 (1.20)
60 to 70	9.33 (1.17)	9.77 (0.32)	8.71 (1.26)	6.31 (1.20)
> 70	10.96 (1.17)	9.55 (1.41)	9.33 (1.45)	6.61 (1.35)

2.6.2.2. Effect of Endurance Training

Endurance training is known to increase aerobic power due to physiological adaptations that improve both oxygen deliver to, and utilisation by, the working muscles. Endurance training is also reported to improve $\dot{V}O_{2\,KINETICS}$ (Hagberg et al., 1980, Berry and Moritani, 1985, Babcock et al., 1994a, Yoshida et al., 1992; Phillips et al., 1995, Norris and Petersen, 1998).

Improvements, after undertaking endurance training, in the $\tau\dot{V}O_2$ of between 8% (Berry and Moritani, 1985) and 58 % (Phillips et al., 1995) have been recorded in males of average age ~ 23 years. The different responses reflect the different training models employed. In a study of older men (mean age 74 years) Babcock et al. (1994a) recorded an improvement of 49 % in the $\tau\dot{V}O_2$ after 24 weeks of endurance training. The $\tau\dot{V}O_2$ (32 s) for the trained older men was similar to the $\tau\dot{V}O_2$ of sedentary younger men (39 s).

Women demonstrated a similar training response to men. $\dot{V}O_{2\,KINETICS}$ improved after a 12 week exercise programme involving women aged 21.6 ± 1.9 years (Paggiosi, 1998). The $\dot{V}O_{2\,KINETICS}$ were measured in the frequency domain using a 15 unit, 30 s duration PRBS protocol.

2.6.2.3. The Effect of Ageing

2.6.2.3.1. Adults

Younger adults generally exhibit faster $\dot{V}O_{2\,KINETICS}$ than both older men (Babcock et al., 1994b; Chillibeck et al., 1996) and older women (Cunningham et al., 1993; Chillibeck et al., 1996). There are, however, difficulties in partitioning out the effects of inactivity or aerobic fitness when investigating the effects of age on $\dot{V}O_{2\,KINETICS}$. In a study by Babcock et al. (1994b) the $\tau\dot{V}O_2$ was shown to be slowed in the older less fit subjects. In order to identify the key factors influencing the slowing of $\dot{V}O_{2\,KINETICS}$ with age, Chillibeck et al. (1996) used a multiple linear regression technique and concluded that relative fitness was the strongest significant influence on $\tau\dot{V}O_2$ as it contributed to 31% of the variance, sex and age were the next most significant indices of $\tau\dot{V}O_2$ in an investigation of young (average age 26.3 ± 2.5 years) compared to old (average age 73.4 ± 12.1 years) subjects of both sexes.

2.6.2.3.2. Children

In two studies investigating the effect of ageing on $\dot{V}O_{2\,KINETICS}$ Freedson et al. (1981) and Macek et al. (1984) included children in the population studied. Although, they reported significant correlations between age and t½, r = 0.77 and 0.69 (Freedson et al. (1981) and Macek et al. (1984) respectively), the model used includes both Phase I and potentially some Phase III components. As Phase I of the response relates to pulmonary blood flow, any differences between adult's and children's blood flow kinetics and consequently, the factors relating to shorter transit times in children compared to adults, will influence the interpretation of the results. Similarly, any contribution of the slower Phase III kinetics will lengthen the t½ of those subjects whose exercise intensity breached the lactate threshold. Closer inspection of the results reported by Macek et al. (1984) suggests that it is the faster $\dot{V}O_{2\,KINETICS}$ of the children that influence the results and therefore the effects of ageing later in life on t½ $\dot{V}O_2$ can be less well established. The research addressing alterations in $\dot{V}O_{2\,KINETICS}$ in children as they grow is considered in more detail in the Section 2.7.

2.7. Oxygen Uptake Kinetics in Children

There are two opposing views relating to the control of $\dot{V}O_{2\,KINETICS}$ in children. The theory postulated by Freedson et al. (1981) suggests that since glycolysis significantly contributes to the energy requirements during the initial minutes of sub-maximum exercise, it can be hypothesised that children's reportedly limited glycolytic capacity will influence sub-maximum $\dot{V}O_2$ dynamics. In opposition to this is the supposition that the rate of the $\dot{V}O_2$ response to exercise is influenced primarily by the need for cellular homeostasis and is age and size independent Cooper et al. (1985).

Early anecdotal and experimental evidence supports the first hypothesis that $\dot{V}O_{2\,KINETICS}$ are faster in children. Robinson (1938) records two groups of boys of mean age 6 and 10.4 years attaining mean values of 55 and 41% respectively of their $\dot{V}O_{2\,MAX}$ during the first 30 seconds of maximal exercise as compared to values ranging from 29 to 35% in adults. Similarly, Astrand (1952) comments that it is probable that younger subjects' ability to adapt themselves more quickly to work than their elders brings about a significant decrease in the amount of anaerobic products (p98), and Godfrey (1974) from observations made in the laboratory states that there is no doubt that children reach a steady state more quickly than adults. The case for children having faster $\dot{V}O_{2\,KINETICS}$ than adults is supported in more recent work by Macek and Vavra (1980a and b), Sady (1981), Freedson et al., (1981); Macek et al., (1984) and Armon et al (1991). A controversy still exists however, as an equal body of evidence (Sady et al., 1983; Cooper et al., 1984; Cooper et al., 1985; Springer et al., 1991; Zanconato et al., 1991 and Hebestreit et al., 1998) demonstrate that $\dot{V}O_{2\,KINETICS}$ remain the same throughout the child's development.

One possible reason why the interpretation of the experimental evidence is equivocal may be a result of the different experimental procedures that have been employed. For example, both high intensity and sub-maximal exercise transitions have been made and both rest to work and work to work transitions have been studied in spite of the fact that $\dot{V}O_{2\,KINETICS}$ are faster with rest-to-work transitions as opposed to work-to-work transitions (Hughson and Morrisey, 1982). These factors will have significant influence

on the interpretation of the results particularly in respect to cross study comparison.

2.7.1. Rest to High Intensity Exercise Transitions

In studies which have compared the $\dot{V}O_{2\ KINETICS}$ of adults and children from rest to maximal or supra-maximal step exercise transitions, the results indicate that children have significantly faster $\dot{V}O_{2\ KINETICS}$ than adults (Robinson 1938; Macek and Vavra, 1980a and 1980b; Sady, 1981). All these studies have reported the whole $\dot{V}O_2$ response without differentiating between the 3 Phases of the response.

2.7.2. Work to High Intensity Exercise Transitions

In the three studies which have utilised high intensity exercise transitions initiated from prior exercise the results are equivocal. Armon et al. (1991) reported that for a spectrum of intensities above the anaerobic threshold the $\tau\dot{V}O_2$ for high intensity exercise was significantly shorter in children than adults for all work rates. Conversely, Zanconato et al. (1991) and Hebestreit et al. (1998) were unable to differentiate between adults and children in terms of differences in the dynamic $\dot{V}O_2$ response. The approach of Zanconato et al., 1991 was unusual in that the protocol was a one minute burst of exercise and therefore comparison with the other two studies may not be appropriate.

The studies of Hebestreit et al. (1998) and Armon et al. (1991) differ in one fundamental respect. Armon et al. (1991) characterise the response by fitting a single exponential plus a linear term to account for the slow component.. The analysis of Hebestreit et al. (1998) only models the Phase II oxygen uptake response with a time delay to account for the Phase I influence. The findings of Armon et al. (1991), support the work of Robinson (1938), Macek and Vavra (1980) and Sady (1981) who also used the same approach of reporting the whole response. The results of Hebestreit et al. (1998) suggest that at the metabolic level the factors controlling cellular respiration are constant throughout growth and they conclude that their results are in contrast to the common beliefs that children rely less on anaerobic energy turnover early in exercise.

2.7.3. Rest to Moderate Intensity Exercise Transitions

The results of studies investigating the changes in $\dot{V}O_{2\ KINETICS}$ during development, in

response to sub-maximal exercise initiated from rest, are again equivocal. In a study of a range of children and adolescents, Freedson et al. (1981) concluded that the mean $t½\dot{V}O_2$ of 34.8 (±12.7) s was not significantly different to adults working at similar intensities reported in other studies. However, regression analysis showed a significant correlation between $t½\dot{V}O_2$ and age (r = 0.77) with the age of the subject accounting for 60% of the reported variance. A significant correlation was also reported of $t½\dot{V}O_2$ with body weight (r = 0.54) and absolute $\dot{V}O_{2\,MAX}$ (r = 0.56). Several possible explanations including body size, maturational level, exercise intensity, anaerobic potential and cardio-respiratory factors were suggested as being associated with the observed relationship between age and $t½\dot{V}O_2$. Similar findings were reported by Macek et al. (1984) who studied the responses of 40 males of different ages (approximate age range 11-40 years). The correlation between age and $t½\dot{V}O_2$ was shown to be highly significant. In a further investigation of 27 boys aged 11-14 years, Macek et al. (1984) reported a significant correlation between body weight and $t½\dot{V}O_2$ ($P < 0.01$). These results suggest the $t½\dot{V}O_2$ response is a function of both workload and age. The influence of increasing $t½\dot{V}O_2$ when operating at high intensities is clearly indicated in the adults studied in this investigation. A comparison of a 10 year old and 40 year old was also made in this study indicating that the child had a faster $t½\dot{V}O_2$ (11.7 s) than the adult (29.1 s). Unfortunately the relative intensity of the work (specifically in relation to the anaerobic threshold) is not given making interpretation difficult.

Conversely, Cooper et al. (1985) could not establish a relationship between age and $\dot{V}O_{2\,KINETICS}$ in 2 groups of younger and older children of age range 7 – 10 and 15 – 18 years respectively. Regression analysis indicated that there was no relationship between $\tau\dot{V}O_2$ and age, weight nor height of the subjects suggesting that the Phase II $\dot{V}O_{2\,KINETICS}$ are independent of development. Similarly, the work of Springer et al. (1988 and 1989) was re-assessed in a 1991 investigation which showed that Phase II $\tau\dot{V}O_2$ was similar in children (27.5 s) and adults (29.9 s) in normoxic conditions. Prior to analysis however, the results were normalised with respect to body weight.

2.7.4. Low to Moderate Intensity Exercise Transitions

In studies of the $\dot{V}O_{2\,KINETICS}$ in children using low to moderate intensity exercise transitions Cooper et al. (1984) calculated the response-time of O_2 uptake (RT-$\dot{V}O_2$) at the onset of an incremental ramp protocol. The RT-$\dot{V}O_2$, although highly variable, was found to be independent of both body size and age suggesting that RT-$\dot{V}O_2$ is not affected by development. This work must be regarded with caution as Hughson and Inman (1986a) and Swanson and Hughson (1988) have argued that a single ramp test is inadequate for determining the time constant of pulmonary $\dot{V}O_2$. The remaining studies that have examined low to moderate intensity exercise transitions (Armon et al., 1991; Zanconato et al., 1991; Hebestreit et al., 1998) have examined the responses to sub-anaerobic threshold exercise as part of studies which have also assessed the high intensity responses. The work of Armon et al. (1991) compared the $\dot{V}O_2$ responses from unloaded cycling to 80%AT in seven adult males, of age range 27 to 40 years, with six children of age range 6 – 12 years. A single exponential model from the start of the transition in work intensities gave a $\tau\dot{V}O_2$ at 80%AT which was significantly quicker in children (26 s) compared to 44 s in adults.

Conversely, Zanconato et al. (1991) found no child-adult differences in the $\dot{V}O_{2\,KINETICS}$ calculated as a $t½\dot{V}O_2$ in response to one minute bursts of exercise at 80%AT. The $t½\dot{V}O_2$ for the 80%AT transition was the same as for the higher intensity exercise undertaken in this study and was not influenced by the size of the work transition in either the children or the adults tested, and there was no significant differences in the mean values between the groups (23.0 ±5.3 s children and 24.8 ± 4.7 s adults). Similarly, Hebestreit et al. (1998) compared the $\dot{V}O_{2\,KINETICS}$ of nine boys with eight men in response to a short exercise protocol of 210 s of exercise at 50% $\dot{V}O_{2\,PEAK}$. An exponential model with time delay was fitted to the $\dot{V}O_2$ responses (Equation 24, Section 2.4.1). There was no significant difference in either time delay nor $\tau\dot{V}O_2$ between boys (22.79 ± 5.05 s) and men (26.4 ± 4.10 s).

From the evidence presented it can be concluded that when the entire response is modelled, children demonstrate faster $\dot{V}O_{2\,KINETICS}$ than adults, however, if the

metabolic response (Phase II $\dot{V}O_{2\ KINETICS}$) is assessed independently, the evidence indicates that adults and children exhibit similar $\dot{V}O_{2\ KINETICS}$.

In summary, it is difficult to ascertain from these investigations if there is a developmental influence on the $\dot{V}O_{2\ KINETICS}$. However, a slightly more coherent picture is emerging that if the whole response is modelled, children exhibit faster $\dot{V}O_{2\ KINETICS}$ than adults in response to both maximal and moderate intensity exercise. When the Phase II $\dot{V}O_{2\ KINETICS}$ is modelled independently the $\tau\dot{V}O_2$ is similar in adults and children.

2.7.5. Limitations of Previous Studies

The conclusions drawn from previous investigations i.e. that there is no difference in Phase II $\dot{V}O_{2\ KINETICS}$ between adults and children, is based upon the assumption that the models used to analyse the responses in adults are applicable to children.

There are also other limitations based on the populations studied. For example, Cooper et al. (1985) suggest that the longer time constants for $\dot{V}O_2$ found in the 15 to 18 year old females compared to younger females aged 7 – 10 years and males aged 7 – 18 years, may be associated with the lower AT/kg and $\dot{V}O_{2\ MAX}$/kg of the older females. Similarly in the study of Hebestreit et al. (1998) although the $\dot{V}O_{2\ MAX}$ was not significantly different between boys and men, it was reasonably high in adults and is perhaps indicative of the adult population being moderately trained (53.4 ± 7.4 and 47.3 ± 5.5 $ml \cdot kg^{-1} \cdot min^{-1}$ in men and boys respectively). As subjects with higher aerobic capacities have consistently been reported to have improved $\dot{V}O_{2\ KINETICS}$ (c.f. Section 2.7.2.1 and Section 2.7.2.2), this factor alone could have resulted in the adult males having faster $\dot{V}O_{2\ KINETICS}$ than might be expected. The influence of fitness on $\dot{V}O_{2\ KINETICS}$ must therefore be considered in the interpretation.

Thirdly, different ages of cohorts have been compared. It is possible that by comparing children with adults of age range 27 - 40 years (Armon et al., 1991), that ageing rather than difference in development has resulted in the children demonstrating significantly faster $\dot{V}O_{2\ KINETICS}$ (c.f. Section 2.7.2.3).

Finally, a range of gas collection techniques have been employed and the majority of the studies reviewed have used manual methods. The poor resolution of the manual method, permitting the collection of respiratory data only every 20 s at best (Zeballos and Weisman, 1994), will reduce the confidence of the estimates of τ and TD due to the limited number of data point around which to construct the model (Equation 24, Chapter 2.4. Section 2.4.1).

3. Variability of Measurements of Oxygen Uptake Kinetics

3.1. Introduction

This chapter serves as a precursor to the materials and methods chapter as it addresses the issues relating to the reliability of measuring $\dot{V}O_{2\,KINETICS}$ and reviews the previous research in this area undertaken within Sheffield Hallam University. The conclusions drawn from this research form the rationale upon which the study described in Chapter 5 is based.

3.2. Measurement Variability

In order to interpret the measurements of $\dot{V}O_{2\,KINETICS}$ with any certainty the experimental method employed must provide results which are reproducible and accurate. Any biological measurements are subject to variability and it is important that the magnitude of the variability of the measurement is known particularly when undertaking comparative studies.

3.2.1. Statistical Methods

Measurement variability has been assessed using a variety of statistical methods (Froelicher et al., 1974; Nordrehaug et al., 1991; Atkinson, 1995).

The coefficients of variation (CV), which is the standard deviation divided by the mean, often multiplied by 100 to give percentage, provides a useful description of the variability of a measure. The problem with this approach is that small means produce large CVs and the calculation of the CV is only appropriate if the measurement error is dependent upon the magnitude of the results (Bland, 1995).

In order to assess the agreement between repeated tests a number of methods are available. The Student's t-test can be used to compare the means of repeated tests or, the analysis of variance (ANOVA), an extension of the t-test can indicate any possible influences of the number of times the test is performed. This may be useful in human physiology to account for the affects of habituation. Other commonly applied statistical techniques to measure the strength of the relationship between test and re-test

measurements are the correlation coefficient (r) or the intra-class correlation (R). Atkinson (1995) concluded that the population characteristics influenced the calculation of both the r and R in such a way as to reduce their reliability, and although these methods provide a measure of the extent of the relationship between two tests, they do not describe how well the two measures agree (Bland and Altman, 1986). An alternative, the "95% limits of agreement" approach has therefore been recommended (Bland and Altman, 1986) as a means to assess the agreement of repeated measures, this method is not affected by population heterogeneity and is recommended as the universal means of assessing the intra-subject variability of replicate test results (Atkinson, 1995). Experimental investigations have been made into the reliability of two different PRBS protocols, a 5 s unit PRBS as described by Bennett et al. (1981) and a 30 s unit PRBS as described by Eßfeld et al. (1987).

3.2.2 Reliability of the 63 unit, 5 s duration, Pseudo Random Binary Sequence Exercise Test to Measure Oxygen Uptake Kinetics

This study was presented as a poster at the First Annual Congress of the European College of Sports Science (Claxton et al., 1996, c.f. Appendix 2.3.)

In a study of the test-retest variability of the 63 unit, 5 s duration PRBS protocol on 20 male subjects (age 26.2 ± 4.0 years) limits of agreement were calculated in order to assess how well individual results obtained in the two tests agreed. The ANOVA showed no significant intra-subject variability between the two tests ($P < 0.05$). Wide 95% limits of agreement were observed at each harmonic for both phase delay and amplitude ratio. This suggests that the 5 s PRBS protocol is reliable for group data, however, the wide limits of agreement indicate a large variability in individual $\dot{V}O_{2\,KINETICS}$.

3.2.3. Reliability of the 15 unit, 30 s duration, Pseudo-random Binary Sequence Test to Measure Oxygen Uptake Kinetics

The results of a reliability study performed using a 15 unit, 30 s duration, PRBS test to measure $\dot{V}O_{2\,KINETICS}$ (Pagiossi, 1998) agree with the findings of the reliability of the 63 unit, 5 s duration, PRBS test.

Eight healthy, moderately active, female subjects (age 22.6 ± 0.8 years, height 1.65 ± 0.06 m and body mass 65.5 ± 4.5 kg) were recruited from the student population at Sheffield Hallam University. An eight (subjects) by two (observations) study design was adopted in order to investigate the variability in $\dot{V}O_{2\,KINETICS}$ in response to the PRBS exercise test. All eight subjects completed two replicate tests in successive weeks. The tests were undertaken on the same day of the week and at the same time of day in order to reduce the effects of diurnal variation on the response (Froelicher et al., 1974).

An assessment of intra-subject variability of the PRBS exercise test was made by means of a two-way ANOVA with repeated measures. An error of linearity test was performed where Pearson Product Moment correlation coefficients were used to assess the extent of the relationship between the measurement error and the measurement size. Where a significant correlation was found, indicating that the error of the measurement was dependent on the size of the result, a CV was calculated. Limits of agreement (95%) were calculated (Bland and Altman, 1986) to provide an assessment of the ability of the PRBS exercise test to repeatedly measure parameters of $\dot{V}O_{2\,KINETICS}$. The 95% limits of agreement method involved the calculation of the mean and the standard deviation of the difference between the replicate measures for both amplitude ratio and phase delay at each of the 4 harmonics.

The results indicated that there were no significant differences in amplitude ratios nor phase delays between each of the four harmonics analysed in Test 1 and Test 2.

Coefficients of variation were not calculated as the error of linearity test showed no significant relationship between the measurement error and the measurement size for either amplitude or phase delay.

The widest 95% limits of agreement (Table 3.1.) for amplitude ratio were 4.952 $ml \cdot min^{-1} \cdot W^{-1}$ which were found at the frequency of 6.7 mHz.

The widest 95% limits of agreement (Table 3.2.) for phase delay were 34.2 degrees which were found at the frequency of 8.9 mHz.

Table 3.1. Mean difference (Test 2 – Test 1) ± 2 × S.D. of the difference between Test 1 and Test 2 and 95% limits of agreement for amplitude ratio ($ml \cdot min^{-1} \cdot W^{-1}$).

	Amplitude ratio (Test 2 – Test 1) (Frequency - mHz)			
	2.2	4.4	6.7	8.9
Mean difference ± 2SD of the difference ($ml \cdot min^{-1} \cdot W^{-1}$)	0.314 ± 0.890	-0.202 ± 0.718	-0.122 ± 0.476	0.052 ± 1.512
95% limits of agreement ($ml \cdot min^{-1} \cdot W^{-1}$)	-0.576 to +1.204	-0.920 to +0.515	-2.598 to +2.354	-1.459 to +1.564

Table 3.2. Mean difference (Test 2 – Test 1) ± 2 × SD of the difference between Test 1 and Test 2 and 95% limits of agreement for phase delay (degrees).

	Phase delay (Test 2 – Test 1) (Frequency - mHz)			
	2.2	4.4	6.7	8.9
Mean difference ± 2SD of the difference (degrees)	-1.1 ± 8.1	0 ± 8.2	-4.8 ± 12.7	1 ± 17.1
95% limits of agreement (degrees)	-9.2 to +7.0	-8.2 to +8.2	-17.5 to +7.9	-16.1 to +18.1

3.2.3.1. Discussion

In assessing variability, the mean difference between measurements obtained in the two tests is important since it is an estimate of the average bias of the measurement. Large mean differences, which would indicate the existence of undesirable trends (Katch et al., 1982), were not detected in this study. A previous study (unpublished data from this laboratory) investigated the reproducibility of the measurement of $\dot{V}O_{2\,KINETICS}$ in four subjects who performed four replicate PRBS exercise tests. A two-way ANOVA with replication did not identify a significant difference between any of the parameters measured during the four tests. Further examination of both individual and mean differences did not identify any bias. It was concluded therefore, that the variability of this study was not due to the nature of the testing procedures or the influence of habituation.

Although no significant difference was found between the replicate parameters of $\dot{V}O_{2\,KINETICS}$ at any of the frequencies assessed, wide limits of agreement, indicating a large degree of variability between the test / re-test results, were evident. This would suggest that it is more appropriate to apply the PRBS exercise test to the study of groups of subjects than to draw conclusions from a single test in individual subjects Other studies have also shown substantial degrees of individual variation in both steady state $\dot{V}O_2$ and $\dot{V}O_{2\,KINETICS}$ (Armstrong and Costill, 1985; Hughson and Inman, 1986a; Nordrehaug et al., 1991; Morgan et al., 1991).

One explanation for the wide intra-subject variability in replicate measures of $\dot{V}O_2$ and $\dot{V}O_{2\,KINETICS}$ is the biological variation or biovariation (Katch et al., 1982). Possible influences of circadian rhythms or psychological factors including anxiety prior to, and during the performance of unfamiliar procedures (British Association of Sports and Science, 1988) may have altered the cardiovascular responses to the PRBS exercise test protocols. The process of removing the initial 450 s of test data in this study should have reduced the effects of any psychological influences due to unfamiliarity with the data collection apparatus and the exercise test sequence.

A second factor which may influence the variability between repeated tests is the inherent breath-by-breath variability (Lamarra et al., 1987). The $\dot{V}O_2$ calculation method employed in this study estimates alveolar gas exchange in accordance with the algorithm of Beaver et al. (1981) this procedure significantly reduces the breath-by-breath variability compared to other techniques which calculate gas exchange at the mouth. In order to further reduce the affects of breath-by-breath variability, irregular breaths caused by abnormal breathing patterns and / or measurement errors, were removed using an algorithm (First Breath Software v2.O, First breath Inc., St Agatha, Ontario, Canada). The three remaining sequences were time aligned and averaged to produce a single mean response, thereby enhancing the underlying physiological signal. To temporally align the $\dot{V}O_2$ responses to the 3 PRBS sequences it was necessary to interpolate the $\dot{V}O_2$ data to produce data points at regular (in this case) 1 s intervals. The number of intermediate data points added to the sequence is therefore dependent on

the breathing pattern of each individual. It was proposed that a greater degree of variability between replicate measures of $\dot{V}O_{2\,KINETICS}$ may have existed for individuals which lower respiratory rates who required the addition of a greater number of data points during the process of interpolation, possibly resulting in distortion of the true response.

3.3. Variability of Oxygen Uptake Kinetics Measurement in Children

No experiments on the variability of gas exchange kinetics measurements have been made on children. Breath-by-breath variability has been suggested as a cause of some of the wide limits of agreement seen in the studies which used adult populations. Close examination of breath-by-breath variability during the PRBS test in a group of adults compared to a group of children showed no difference between the two groups. It is expected that the reliability measures for the children would be similar to those reported for adults (Kusenbach et al., 1994).

3.4. Conclusions from the Variability Studies

- The individual variability of the PRBS exercise test has been shown to be considerable for both young adult male and female subjects.
- The results from several repetitions of the PRBS exercise test should be pooled to give a more reliable estimate of $\dot{V}O_{2\,KINETICS}$ for any individual subject.
- The test could be usefully applied to the assessment of group $\dot{V}O_{2\,KINETICS}$.
- Techniques need to be applied whereby breath-by-breath variability is reduced prior to interpolation of the $\dot{V}O_2$ data.

Mathematical techniques such as cross-correlation may further improve the confidence in the measurement of $\dot{V}O_{2\,KINETICS}$ by elimination of anomalous $\dot{V}O_2$ responses.

4. Materials and Methods

As the methodology employed in the investigation detailed in Chapter 5 of this thesis is fairly complex, Chapter 4 has been included as a practical description of the materials and methods used in the final investigation and is divided into three sections. Section 4.1 relates to the equipment used, Section 4.2 describes the protocols employed and the methods of data analysis, and Section 4.3 defines the three methods used to express economy and efficiency of work.

4.1. Equipment

The equipment used for experimentation is described in five sections: cycle ergometry, mass spectrometry (including calculations of alveolar gas exchange), electro-cardiography, anthropometry (including maturity assessments) and spirometry.

4.1.1. Ergometry

The cycle ergometer (550 ERG, Bosch, Berlin, Germany) was selected to provide the exercise stimulus as the PRBS method and the calculation of oxygen cost of work from the ramp protocol require an exact determination of the work rate. The Bosch 550 ERG cycle ergometer used in this study is loaded using an eddy current brake which is adjustable from unloaded to 400 W either manually in 5 W steps, or electronically by a control system via a serial interface. An electronic circuit ensures that the work required of the subject remains constant independent of pedal speed.

The data of Maxwell et al (1998) illustrates the need to evaluate the calibration of electromagnetically braked cycle ergometers, as the individual accuracy of the four different machines they tested, ranged from 89.3 to 101.4% over the up-scale range of 100-400 W. Part of the variability in the accuracy data presented by Maxwell et al (1998) was attributed to the age of the ergometer and to factors relating to servicing. These findings emphasise the need for cycle ergometers to be calibrated using a dynamic calibration rig (DCR) on a regular basis if valid measures of power output are required.

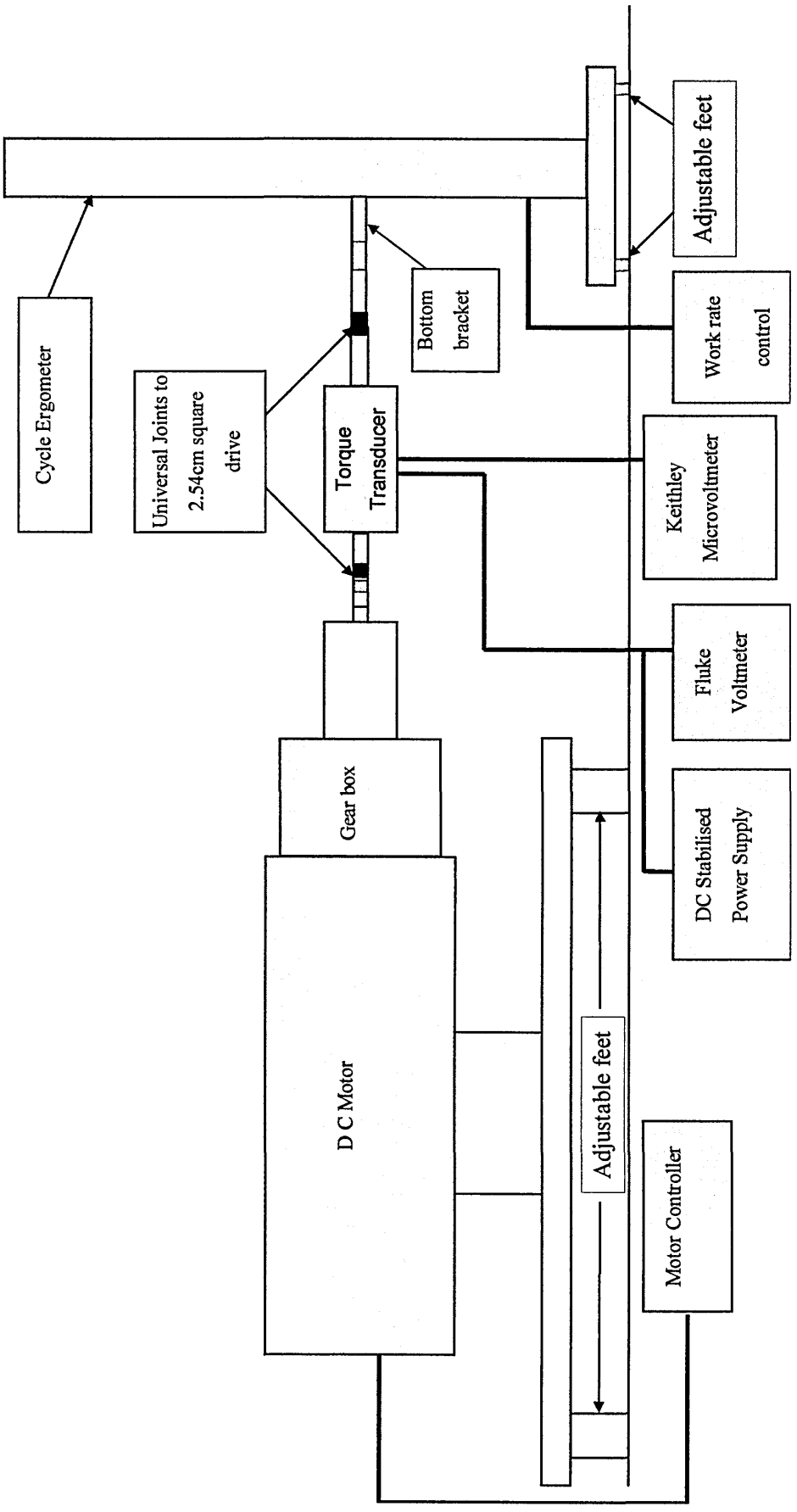


Figure 4.1. Configuration of the dynamic calibration rig.

4.1.1.1. Dynamic Calibration Rig.

The cycle ergometer used in this study was calibrated using a DCR constructed by the Medical Physics Department, Royal Hallamshire Hospital, Sheffield (Figure 4.1). The components of the DCR consisted of a rotary torque transducer TRSC-100 with a range of 100 Nm (Industrial Measurements Limited, Derby, England), coupled between the cycle ergometer crank axle (bottom bracket) and a 0.75 kW motor with Spaggiari gear assembly. The speed of the motor was governed using a Eurotherm DC Motor Controller (Model 503-1.25 HP). Both the Motor and Controller were supplied by Beatson Fans and Motors, Sheffield, England.

Measurements were recorded form the torque transducer using two digital voltmeters (Fluke Model 75 which was cross calibrated with a Keighly 197 Autoranging Microvoltmeter). The transducer was stabilised using a direct current (DC) stabilised power supply (Weir Minireg type 402). The microvoltmeter and power supply were supplied by RS components (Corby, Northamptonshire, England.)

The accuracy of the torque transducer was determined using free weights and found to better than ±1%. The microvoltmeter is rated to an accuracy within ±16 microvolt and the speed controller, which was used in voltage feedback mode attained speed accuracy of within ± 2%.

4.1.1.2. Calculation of Mechanical Power

Actual power was calculated using the following formula :

$$P = T\omega \quad \text{.....(28)}$$

Where P is power (in Watts) and T is torque (in Newton meters). Angular velocity (ω) is 360 degrees or 2π radians for one revolution.

$$P = T2\pi(n/60) = 0.1047Tn \quad \text{.....(29)}$$

Where n is the speed of rotation in revolutions per minute. With a 10 volt bridge rotary :

$$\text{Torque} = mV/0.17 \quad \text{.....(30)}$$

By substituting (30) into (29),

$$P = 0.6159 \times n \times mV \quad \text{.....(31)}$$

4.1.1.3. Calibration Procedure

The bottom bracket of the cycle ergometer and the DCR were aligned using adjustable feet to ensure minimal movement of the assembly during operation. The motor was adjusted to maintain a constant rotation of 1Hz throughout the evaluation. The First Breath Inc. (Marquette Electronics Inc. Milwaukee, USA) v 2.0 Work Rate Control Software was used to programme the cycle ergometer to operate from unloaded then increasing in power to a maximum of 400 W in 25 W increments. The same sequence was repeated in reverse order to assess for any differences in performance when reducing as opposed to increasing work intensities were induced. Additional measurements were made at 20 W, 30 W and 80 W to enhance the lower range assessment. Each work load was maintained for two minutes and the output from the torque transducer was sampled using the microvoltmeter at a frequency of 1Hz during the last 20 seconds of each intensity.

4.1.1.4. Calibration curves

Comparison of the up- and down- scale work rate measurements were made using a calibration curve (Figure 4.2).

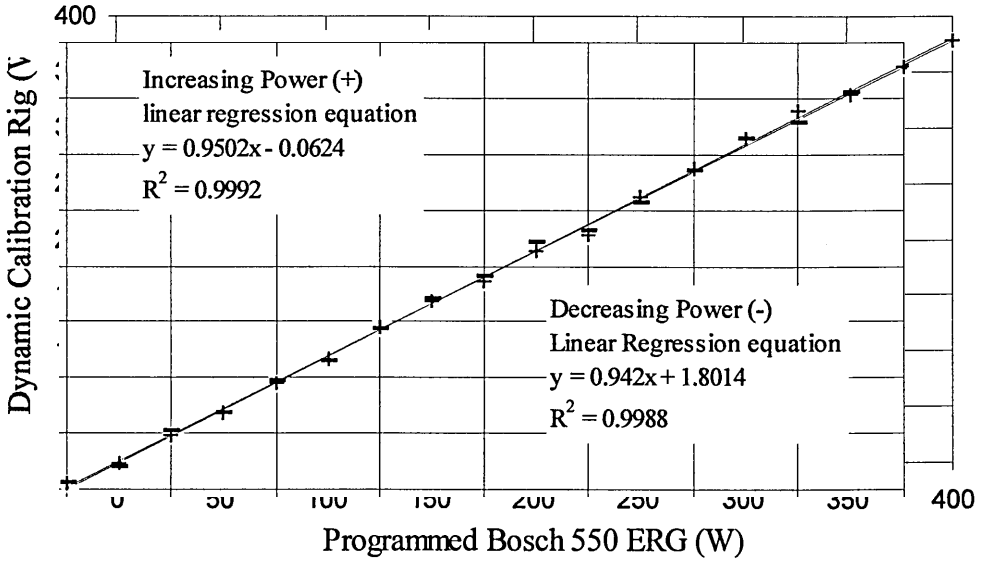


Figure 4.2. Calibration curve for independent upscale and downscale measurements of the dynamic calibration rig (y-axis) and programmed Bosch 550 ERG (x-axis).

As the data collected upscale and downscale were similar (no evidence of hysteresis), the 40 measurements made at each intensity were pooled to construct appropriate calibration curves for the full range of work intensities used in the ramp test to determine $\dot{V}O_{2\,MAX}$ (Figure 4.3) and for the range of work rates required for the PRBS test (Figure 4.4).

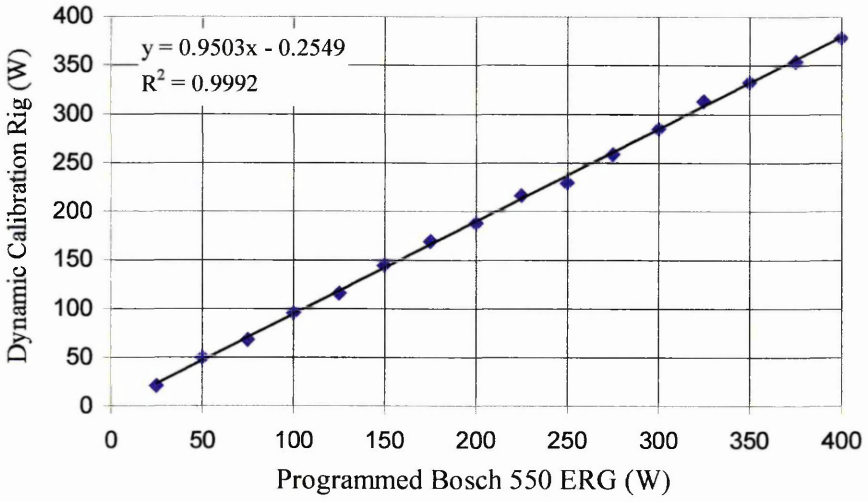


Figure 4.3. Calibration curve for the full range of work rates required for the maximum aerobic capacity test.

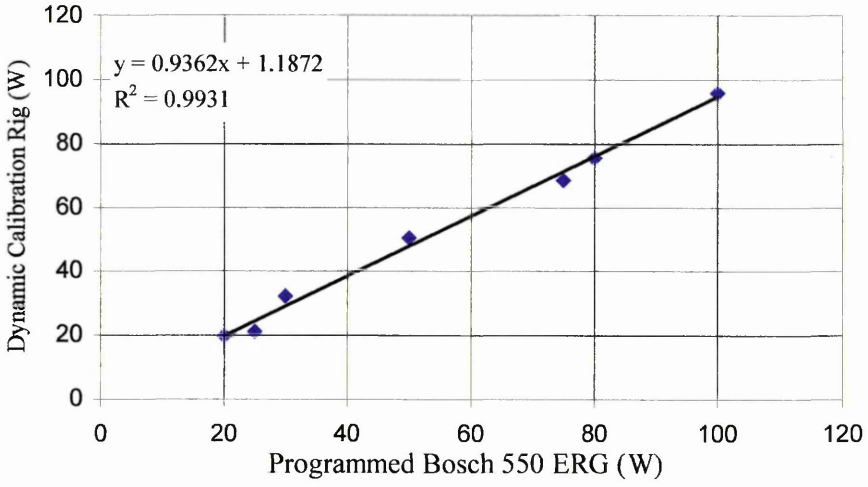


Figure 4.4. Calibration curve for the range of work intensities required for the PRBS test.

The high R^2 in Figure 4.3 shows the ergometer to be linear throughout the range. The actual work rate is lower than the programmed value at a rate of 0.5 W for every 10 W increment across the range 25 to 400 W. This relates to an accuracy of – 5% at 400 W,

which is in line with the manufacturers specification of an accuracy of ± 5% over the range 60-400 W (Bosch ERG 550 Instruction Manual, 1990).

From the low intensity calibration equation (Figure 4.4) the actual power at 60 W was 57.4 W, which is 2.6 W below the programmed value. This is within the manufacturers specified accuracy of ± 3 W over the range 25-60 W (Bosch ERG 550 Instruction Manual, 1990).

All subsequent work intensities used in the PRBS and $\dot{V}O_{2\,MAX}$ tests were calculated from the corrected values derived from the low intensity and the full range calibration curves respectively.

4.1.1.5. Cycle Ergometer Work Rate Response Time

As the PRBS protocol depends upon rapid square wave changes in work rate, the speed of change in the cycle ergometer power was measured by monitoring the DC input signal to the cycle ergometer, from the control software, and the DC output response from the cycle ergometer, using a flat bed recorder (Linseis, GMbH LS/L600/L650, Scientific and Medical Products Ltd, Didsbury, UK). The input signals were confined to those work rate changes to be used in the tests for $\dot{V}O_{2\,KINETICS}$ i.e. 20–50 W, 25-80 and 25-85 W. Both upscale and downscale recordings were made between each of the three intensities.

The maximum response time for all intensities was observed to be within 1 s.

4.1.2. Respiratory Gas Analysis Apparatus

The respiratory gas analysis system used as the tool for the evaluation of oxygen uptake kinetics was selected using the following criteria.

Initially, it is largely accepted that mass spectrometry is the most accurate method of gas analysis available (Clausen, 1982). Secondly, the use of the mass spectrometer as a reference system in order to evaluate other respiratory analysis systems (Wilmore and Costill, 1973b; Wilmore et al., 1976; Jones, 1984) indicates its acceptance as an accurate method for respiratory gas analysis. Thirdly, the ability to measure nitrogen and estimate alveolar gas exchange has been recommended for studies of the kinetics of

gas exchange (Beaver et al., 1981), and finally, in a comparative evaluation with another respiratory system available for the study (Appendix 4.1), the respiratory mass spectrometer compared favourable with normative steady-state data indicating the validity of its measurement.

4.1.2.1. Respiratory Mass Spectrometry

Measurements of inspired and expired respiratory gas concentrations were made using a respiratory mass spectrometer (MGA-1100, Marquette Electronics Inc., Milwaukee, WI, USA.). Breath-by-breath inspiratory and expiratory gas flows were measured using a bi-directional flow turbine (VMM-2, Interface Associates, Laguna Niguel, CA,USA) The ventilatory volume is determined indirectly as the integral of flow against time. The ventilation and gas concentration values were digitally sampled at a frequency of 200 Hz. Four of the primary analogue signals generated by the mass spectrometer and ventilation system in response to several breathing cycles are shown in Figure 4.5.

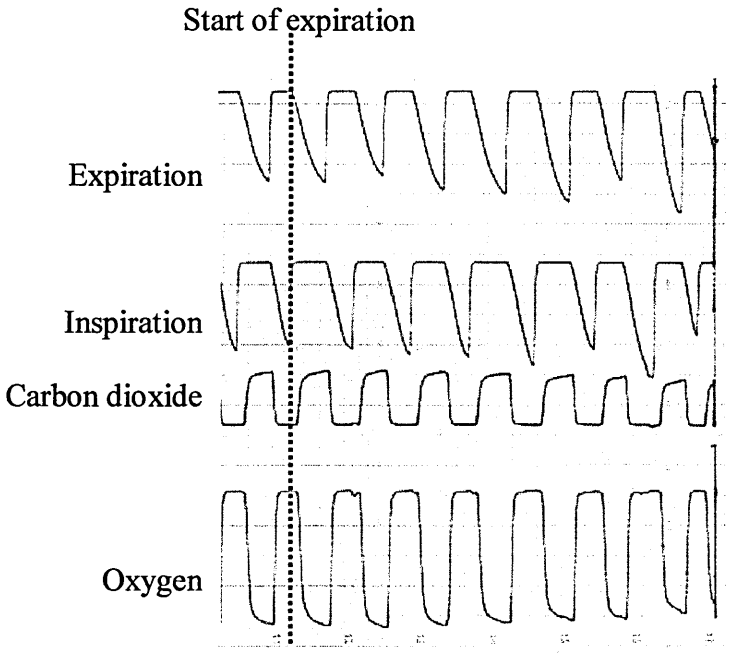


Figure 4.5 The analogue signals (CO_2 and O_2) generated by the respiratory mass spectrometer in relation to the ventilation signal generated by the flow turbine.

The signals from the mass spectrometer and flow measurement system were interfaced with a PC-compatible desk top computer (Ti'ko PS 325C, Ti'ko Computer Corporation,

Broxburn, UK.) via an analogue to digital converter. The signals were integrated on-line, using First Breath Software v2.0 (First Breath Inc., St Agatha, Ontario, Canada, 1992). The system provides estimates of alveolar gas exchange based on the algorithm of Beaver et al. (1981).

4.1.2.2. Correction of Gas Volumes

When making comparisons between tests carried out under different atmospheric conditions it is necessary to apply a correction factor to account for the effects of ambient temperature, pressure and water vapour on the measured volumes. The universally accepted method is to express the measured volumes in terms of either, Standard Temperature and Pressure Dry (STPD), or Body Temperature and Pressure Saturated (BTPS). Standard temperature and pressure dry is used for all metabolic calculations including $\dot{V}O_2$ and is calculated as a dry gas at a temperature of 273 K and a pressure of 760 mmHg. When referring to variables such as $\dot{V}E$, BTPS is used, that is a temperature of 310 K, ambient pressure and saturated with water vapour of partial pressure of 47 mmHg which is the vapour pressure of water at body temperature of 37° C (Fox et al., 1993).

4.1.2.3. Calibration of the Respiratory Gas Analysis System

The gas flow turbine (Figure 4.6) was calibrated prior to each test using a 3 litre calibration syringe (Hans Rudolf Inc., Kansas City, MO, USA). The calibration syringe was operated in a pulsatile manner at a rate of approximately 2 $l \cdot s^{-1}$. This rate was selected as a standard approach to maximal and sub-maximal testing in both adults and children to ensure consistency. This method has provided volume measurement of similar accuracy to pneumotachometers calibrated at higher rates assessed during low and moderate intensity exercise (Appendix 4.1). The accuracy of the turbine volume determination was deemed to be suitable if the average of 5 inspiratory and 5 expiratory excursions was within 1% of the actual value (i.e. ± 30 ml).

The gas flow turbine has a low-dead-space (90ml) which is critical when performing metabolic measurements on children as they have relatively low tidal volume. This

corresponds with the method used by Cooper et al. (1985) in a similar study who used a valve with a 90 ml dead-space for male and female subjects aged 7-10 years and above. The respiratory mass spectrometer was calibrated using two cylinders of calibration gas certified to be accurate to ± 0.03 volume percent (Medical Graphics Corporation, St Paul, MN, USA). Cylinder 1 containing 12% O_2 and 5% CO_2, and cylinder 2 containing 21 % O_2, the balance of both cylinders made up of N_2. The two cylinders enabled a two point calibration, 0 and 5% for CO_2, and 12% and 21% for O_2. A measurement tolerance of ± 0.05% of the cylinder gas concentration was permitted prior to re-calibration of the mass spectrometer. At no time during the course of the experimentation, was this tolerance exceeded indicating exceptionally high stability of the mass spectrometer. Partial validation of the calibration was determined by sampling atmospheric air from an external window - the system consistently returned values of 0.03-0.04% CO_2 and 20.93 O_2.

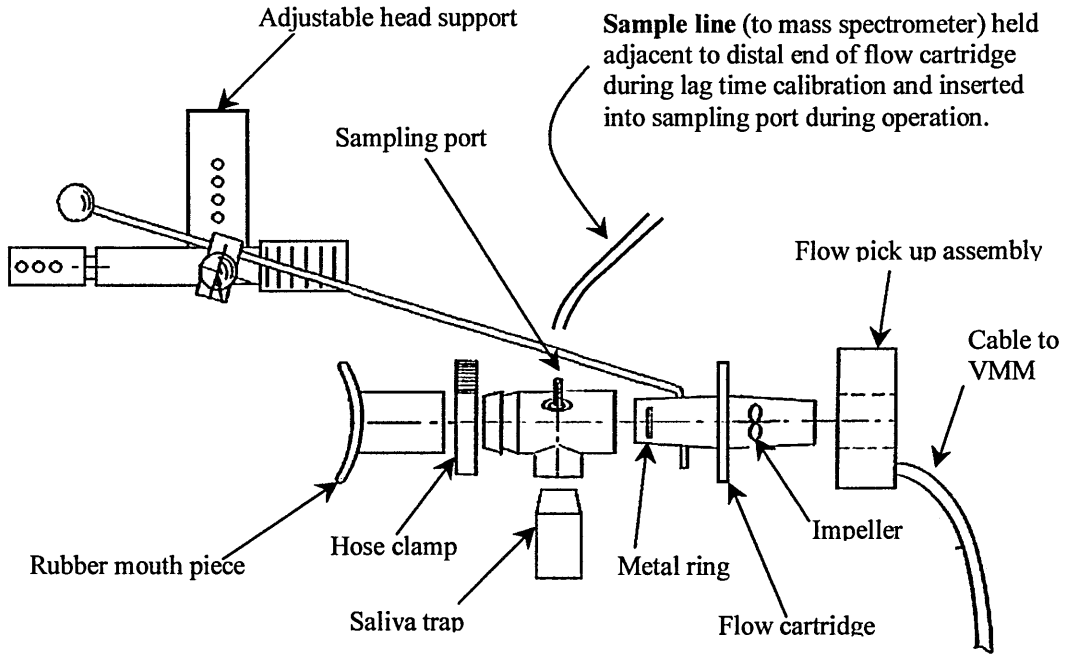


Figure 4.6. Gas flow turbine assembly.

4.1.2.4. Alignment of analysis signals

An algorithm (First Breath software v2.0, First Breath Inc., St Agatha, Ontario, Canada, 1992) to determine τ (the time constant for the exponential rise in measured CO_2 concentration) was used to calculate the delay or lag time between the signal change at the start of inspiration, detected by the turbine flow meter, and the rise time in the concentration in the CO_2 signal, measured by the mass spectrometer. Alignment of the volume signal with the gas concentration signal was carried out by performing a breathing manoeuvre to introduce a square wave change in the CO_2 signal. This was achieved by slow exhalation into the gas flow turbine assembly followed by rapid inhalation. This procedure was performed with the sample line (Figure 4.6) held adjacent to the distal end of the assembly in order to improve the speed of transition between expired and ambient CO_2 during the inspiration phase.

This breathing manoeuvre was carried out prior to and immediately after each exercise test. Lag time was found to be stable at 220 ms throughout all studies. The time lag between the ventilation signal which occurs almost instantaneously and the changes in gas concentration can be identified in Figure 4.5.

4.1.2.5. Estimation of Alveolar Gas Exchange

The Marquette mass spectrometer, Alpha Technologies turbine and the First Breath v2.0 software (First Breath Inc., St Agatha, Ontario, Canada, 1992) was used to derive a breath-by-breath estimation of alveolar gas exchange based on the algorithm of Beaver et al (1981). This method requires an estimate to be made of effective lung volume (ELV). The initial estimate for the ELV was made using a volume equal to half the estimated functional residual capacity (½FRC) obtained from normal tables which take into account the height, gender and age of the subject.

To minimise the inter-breath variability, a post hoc calculation of ELV was made as described below and in Appendices 4.2 and 4.2a.

1) calculate difference in $\dot{V}O_2$ between each successive breath

2) square this difference

3) sum the squared values

4) use Excel Solver (Microsoft Excel 97 SR-1, Microsoft Corporation, Redmond, WA, USA) to calculate the lung volume which effectively minimises the sum of squares value thereby reducing the inter-breath variability (Swanson, 1980).

Subsequent breath-by-breath estimates of alveolar gas exchange are made using the post hoc calculated value of ELV in the following formula:

$$\dot{V}O_2 = [(VIO_2 - VEO_2) - (\Delta VL \times FetO_2) - (\Delta FetO_2 \times ELV)] \times fb$$

where

$$\Delta VL = [(VN_2 - ELV) \times \Delta FetN_2]/FetN_2$$

Refer to Chapter 2 Section 2.3.3 for key to abbreviations or the table of abbreviations in the contents pages.

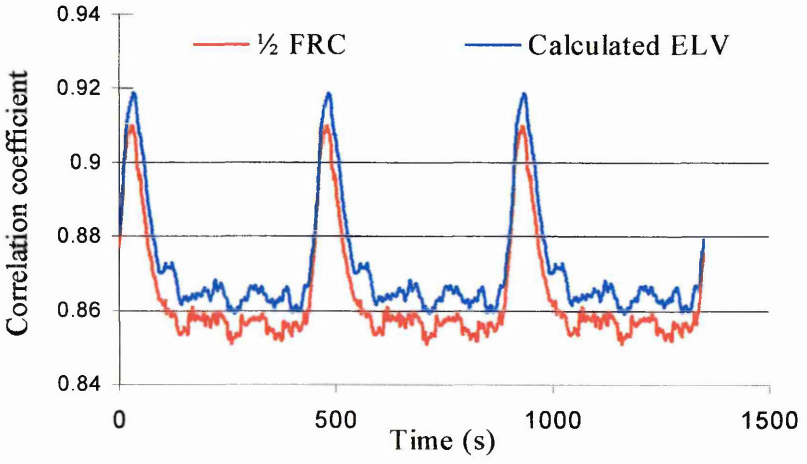


Figure 4.7. Power spectrum showing cross-correlated (oxygen uptake-work rate) profile for three repetitions of a 450 s PRBS exercise test. The cross correlations shown are based on a oxygen uptake computation using NLV equivalent to ½ FRC and the post-hoc calculation of ELV. The maximum correlation coefficient of a PRBS signal repeats at intervals equivalent to the duration of the pseudo-random sequence.

The algorithm of Beaver et al. (1981), accounts for changes in pulmonary gas stores on a breath-by-breath basis and this method has been shown to have a flow meter error sensitivity of less than one (Swanson et al., 1981). The sensitivity to expiratory error can be quite large in traditional methods which do not correct for changes in pulmonary gas stores, the approach, shown above is therefore the method of choice for gas exchange studies to transient exercise.

By using the least squares post–hoc analysis to minimise breath-to-breath variation the signal to noise ratio can be improved further. This can be seen as an elevated cross-correlation profile in Figure 4.7.

4.1.2.6. Filtering of the Oxygen Uptake Data to Remove Noise

Prior to any analysis unusual (non physiologic) $\dot{V}O_2$ data were removed from the data record. The method used to identify the non-physiologic $\dot{V}O_2$ data was to calculate the mean breath-to-breath change in $\dot{V}O_2$ and, any breath-to-breath variation in $\dot{V}O_2$ greater or less than 3 standard deviations of the mean breath-to-breath difference were identified and removed. Different smoothing techniques were then employed for each the different variables assessed :-

For detection of T_{vent} a 3 breath rolling average was used.

For the expression of variables at $\dot{V}O_{2\,MAX}$, the data was presented as 30 second average values.

For the calculation of $\dot{V}O_{2\,KINETICS}$, $\dot{V}O_2$ values were linearly interpolated between breaths to yield values every one second. This provided data at regular intervals for subsequent analysis. The basic steps of the noise reduction technique used in the $\dot{V}O_{2\,KINETICS}$ analysis are shown in Figure 4.8.

The auto-correlation of the PRBS work rate input (ACF) and the cross-correlation function (CCF) between the work rate input and $\dot{V}O_2$ output are calculated in order to remove the effects of $\dot{V}O_2$ values which are uncorrelated with the exercise response.

The computer program used to calculate the correlation profiles shown above are described in Appendix 4.3.

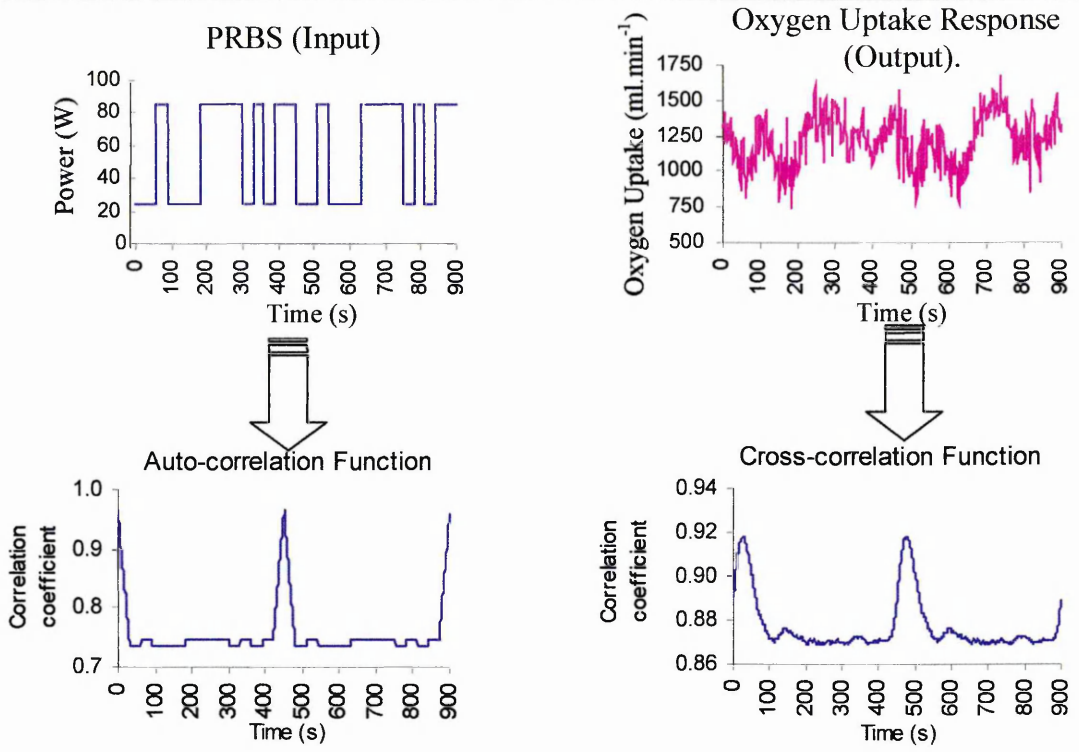


Figure 4.8. Noise reduction procedure. Calculation of the auto-correlation function of the input (Power) and cross-correlation function of the output (Oxygen Uptake) for two pseudo-random binary sequences.

4.1.2.7. Steady State Oxygen Uptake Measurements

Prior to measuring the dynamic $\dot{V}O_2$ response to exercise biological calibrants were used to assess the steady state $\dot{V}O_2$ responses reported by the Marquette system. The findings (Appendix 4.1) demonstrate that the Marquette gas analysis system measures $\dot{V}O_{2\,(ss)}$ which is consistent with that reported by other researchers.

4.1.3. Electrocardiography

A 10-lead digital electrocardiogram (ECG) (CASE 15, Marquette Electronics Inc., Milwaukee, WI., USA.) was used to record cardiac frequency continuously throughout all tests. The ECG was interfaced with the respiratory analysis computer via an serial (RS232) connection. Lead II alone was chosen to monitor cardiac frequency and the standard bi-lateral, sub-clavicular and 5th intercostal space electrode configuration was used. Prior to placement of the Ag/AgCl diaphoretic monitoring electrodes (Red Dot, 3M, London, Ontario, Canada) the skin was prepared using an abrasive pad and ethanol wipes to ensure good electrical contact.

4.1.4. Anthropometry and Maturity Assessments

The height (in m to the nearest 0.01 m) and body mass (in kg to the nearest 0.1 kg) of each subject were measured using a combined stadiometer with beam scale (Avery, Birmingham, England).

Sexual maturation of the child subjects was determined by a paediatric clinician using a 5 point scale (Tanner, 1962). A Tanner rating of 1 is the pre-pubertal classification and stage 5 is sexually mature. The Tanner score was generated using the average score for pubic hair rating and genitalia (boys) or breast rating (girls).

4.1.5. Spirometry

The children's lung volume measurements were made using a Morgan eight litre "TLC test" rolling seal dry spirometer (P.K. Morgan Ltd., Chatham, UK). Each child received a short period of instruction from an experienced technician immediately before testing; tests were performed with the children standing and wearing nose clips. Forced expiratory variables (forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were determined from the best of three efforts (Quanjer et al., 1993).

4.2. Exercise Protocols

All subjects performed two tests: an incremental ramp protocol for the determination of ventilatory threshold (T_{vent}), maximum aerobic capacity ($\dot{V}O_{2\,MAX}$) and oxygen cost of cycle ergometer exercise, and a sub-maximal pseudo-random binary sequence (PRBS) exercise test for the determination of $\dot{V}O_{2\,KINETICS}$.

4.2.1. Incremental Ramp Protocol

Each subject performed a continuous, incremental, ramp protocol to maximum volitional exertion on the cycle ergometer in order to assess aerobic function (Whipp et al., 1981). Prior to starting the test, seat height and handlebars were individually adjusted and each subject was allowed a period of familiarisation with the respiratory apparatus and ergometer.

To account for the variation in age, height and weight of the test population, 3 different ramp protocols were used to ensure that the test would terminate after approximately 6-

14 minutes of cycling (Cooper et al., 1990).

The standing height of the children was used as the criterion for determining the rate of increase in intensity appropriate for use in these children. Godfrey (1974) recommends a 15 $W \cdot min^{-1}$ ramp protocol for children in the height range 1.25 –1.50 m. It was expected that this approach would result in the completion of the test by the majority of children in a maximum of 9 minutes.

The children's ramp protocol commenced with unloaded cycling (approx. 5 W), after which the workload increased continuously at a rate equivalent to 15 $W \cdot min^{-1}$. For the adult females, the protocol commenced with 2 minutes of work at 40 W followed by work increments equivalent to 20 $W \cdot min^{-1}$. The adult males commenced the protocol with 2 minutes of cycling at 75 W followed by a 25 $W \cdot min^{-1}$ ramp protocol. Subjects maintained a pedal frequency of 1 Hz throughout the exercise. Strong verbal encouragement was used to motivate the subjects during the latter stages of the test, which terminated when the subjects were unwilling or unable to maintain a pedal frequency of 0.66 Hz (Hughson et al 1991c).

The use of different protocols for each group resulted in test durations of 6.3-15.3 minutes.

After completing the progressive exercise test to exhaustion, all subjects performed a 5 minute cool down on the cycle ergometer.

4.2.1.1. Determination of Maximum Oxygen Uptake

Maximum oxygen uptake was said to have been achieved during the progressive exercise test when there was no further increase in oxygen uptake despite further increases in work intensity (Astrand and Rodahl, 1986). In some subjects the characteristic plateau in the $\dot{V}O_2$ response could be discerned when the subject continued to exercise for at least one minute above the work intensity that elicited $\dot{V}O_{2\,MAX}$. In other subjects a true plateau was not attained but was considered as a plateau when the change in $\dot{V}O_2$ measured over the final minute of exercise was less than 2 standard deviations below the mean increase in $\dot{V}O_2$ measured during 4 or 5

minutes of sub-maximal progressive exercise (Rowland and Cunningham, 1992).

As the criteria for $\dot{V}O_{2\,MAX}$ described above was rarely satisfied, for confirmation that the highest oxygen uptake attained at the termination of the progressive exercise test to exhaustion can confidently be accepted as a maximal indices, additional criteria (Figure 4.9) were considered.

As the response to maximal exercise is different in children and adults it was necessary to adopt different criteria for the two groups.

Figure 4.9. Additional criteria for determination of $\dot{V}O_{2\,MAX}$ in Adults and Children

At test termination the subject must show signs of intense effort and additionally exhibit one or more of the following :

In adults :-

- heart rate 95% of maximal heart rate predicted for age.

  Age predicted maximum heart rate = (220 – age)
  (American College of Sports Medicine,1991)

- respiratory exchange ratio (RER) ≥1.15 (Zeballos and Weisman 1994).

In children :-

- heart rate to begin to plateau at a value at least 190 beats·min^{-1}(Rowland et al. 1997)
- An RER_{MAX} of ≥ 1.0 (Rowland et al. 1997)
- $\dot{V}E_{MAX}$ = 60% predicted Maximum Voluntary Ventilation (pMVV)

  pMVV = *FEV_1× 35
  (Godfrey, 1974, Armstrong et al., 1997)

*FEV_1 is Forced expiratory volume in 1 second (refer to section 4.1.5.).

4.2.1.2. Determination of Ventilatory Threshold

The ventilatory threshold (T_{vent}) was determined from the breath-by-breath gas exchange data collected during the incremental exercise test to exhaustion. Individual graphs of $\dot{V}E/\dot{V}O_2$, $\dot{V}E/\dot{V}CO_2$, end tidal O_2 ($PETO_2$) and CO_2 ($PETCO_2$) were plotted against work rate (Wasserman et al., 1987) (Appendix 4.4). Additionally $\dot{V}CO_2$ were plotted against $\dot{V}O_2$, (V-slope plot) (Appendix 4.4a) in accordance with Beaver et al. (1986). The initial method to detect T_{vent} was to visually inspect the $\dot{V}E/\dot{V}CO_2$ graph and locate the respiratory compensation threshold (RCT). The RCT is the point at which

$\dot{V}E$ increases out of proportion to the increase in $\dot{V}CO_2$ thereby providing ventilatory compensation for the exercise induced lactic acidosis (Beaver et al. 1986). The RCT provides a cut off point above which the data is excluded from further evaluation. The T_{vent} was then located by finding the lowest point (nadir) of $\dot{V}E/\dot{V}O_2$ and $PETO_2$, before they began to increase consistently without a concomitant increase in $\dot{V}E/\dot{V}CO_2$ or a decrease in $PETCO_2$ (Whipp et al., 1981). The T_{vent} represents the point at which $\dot{V}E$ increased out of proportion to the increase in $\dot{V}O_2$ (hyperventilation with respect to O_2). Hyperventilation with respect to O_2 without concomitant hyperventilation for CO_2 only occurs during buffering of a metabolic acid by HCO_3^-. Other forms of hyperventilation should cause $PETO_2$ to increase and $PETCO_2$ to decrease while $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ increase together. Final confirmation that the T_{vent} had been identified accurately was sought by looking for the 'break point' in the relationships between $\dot{V}CO_2$ when plotted against $\dot{V}O_2$ (the V- slope method - Beaver et al., 1986).

4.2.2. Pseudo-random Binary Sequence Exercise Test Protocol

Following a warm up period, the subjects completed a single PRBS exercise test. The PRBS test consisted of two (for the children) or three (for the adults) identical sequences. Each sequence consisted of fifteen 30 s units. Throughout each test the work rate was automatically switched between 20 and 50 W for the children, 25 and 85 W for the adult males, and 25 and 80 W for the adult females (Table 4.1).

Table 4.1. The protocol for a single 450 s pseudo-random binary sequence.

	Duration at each intensity (s)							
PRBS Protocol	60	30	90	120	30	30	30	60
Children (W)	20	50	20	50	20	50	20	50
Adult male (W)	25	85	25	85	25	85	25	85
Adult female (W)	25	80	25	80	25	80	25	80

These work rates were selected to maximise the signal to noise ratio without compromising the dynamic linearity of the system. All exercise was performed at a pedalling frequency of 1 Hz.

4.2.2.1. Oxygen Uptake Analysis in the Frequency Domain.

The ACF and CCF of the input and output were transformed from the time domain into the frequency domain using Fourier analysis (Appendix 2.2). The relationship between the ergometer input and $\dot{V}O_2$ output was calculated as an amplitude ratio ($ml \cdot min^{-1} \cdot W^{-1}$) and a phase shift (degrees) as follows :-

Amplitude ratio = $\dot{V}O_2$ Amplitude / Work rate amplitude

Phase Shift (Degrees) = Work rate phase angle - $\dot{V}O_2$ phase angle

Only those parameters in the frequency range 2.2- 8.9 mHz were considered suitable for statistical analysis (Hoffman et al. 1994b).

4.2.3. Oxygen Cost of Cycling

The oxygen cost of cycling was calculated from both a) the progressive exercise test and b) the PRBS test.

4.2.3.1. The Progressive Exercise Test

Work economy was calculated using least squares regression analysis to derive a formula to describe the relationship between work rate (W) and oxygen uptake ($ml \cdot min^{-1}$) (Figure 4.10).

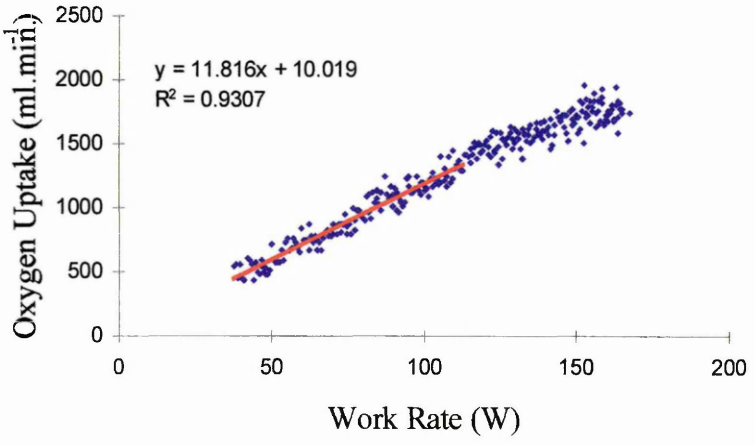


Figure 4.10. Progressive exercise test performed by a 13 year old male. The regression curve, describing the relationship between oxygen uptake and work rate, is fitted to data collected between the start of the second minute of exercise and the time at which 90% T_{vent} was attained.

Delta efficiency (%) was estimated by substituting the gradient derived in the above regression analysis into the following equation :-

$$\Delta\text{efficiency (\%)} = \frac{291}{\text{gradient}}$$

This efficiency estimation is based on the assumption of a constant RER of 0.9.

The analysis for the above calculations was restricted to moderate intensity exercise by discarding the $\dot{V}O_2$ and work rate data during the first minute of the ramp protocol and analysing the data between the start of the second minute and the time at which 90% T_{vent} was attained.

4.2.3.2. The Pseudo-random Binary Sequence Test.

The amplitude ratio of harmonic 0 from the Fourier analysis performed on the PRBS data, represents the average $\dot{V}O_2$ and W relationship for the whole test. Unlike the calculation of work economy calculated from the response to the ramp protocol, which relates solely to the oxygen cost of work, the amplitude ratio of harmonic O includes the oxygen requirement at rest which will be different between individuals of different size.

5. A Comparison of the Oxygen Uptake Kinetics of Adults and Children

5.1. Introduction

In healthy individuals, Phase II $\dot{V}O_{2\,KINETICS}$ have been shown to be affected by physical fitness (Hagberg et al., 1980; Powers et al., 1985; Eßfeld et al., 1987; Zhang et al. 1991), endurance type training (Berry and Moritani 1985; Babcock et al. 1994a; Phillips et al., 1995; Yoshida et al., 1992; Edwards et al., 1999; Norris and Petersen, 1998) and by factors relating to ageing (Babcock et al. 1994b; Chillibeck et al., 1996 and 1997).

These effects may be a result of changes in the oxygen delivery system or reflect peripheral changes associated with enhanced oxidative metabolism.

It is surprising therefore, that, considering children's enhanced aerobic metabolism (Eriksson, 1972; Eriksson, 1980; Bell et al., 1980; Haralambie, 1982; Taylor et al., 1997) and reduced glycolytic flux (Bar-Or, 1983; Berg et al., 1986; Zanconato et al., 1993), the evidence for children demonstrating improved $\dot{V}O_{2\,KINETICS}$ in comparison with adults is equivocal.

Only two groups of researchers (Springer et al., 1991; Hebestreit et al., 1998) have specifically investigated the Phase II $\dot{V}O_{2\,KINETICS}$ differences between children and adults using the modelling technique currently deemed appropriate (Chapter 2, Section 2.4.1). Both of these groups of researchers have found $\dot{V}O_{2\,KINETICS}$ to be the same in children and adults. In both these studies, however, the use of different age ranges and the difficulties associated with accounting for the influence of fitness parameters may have influenced the interpretation of the results.

The aim of this study was to compare the $\dot{V}O_{2\,KINETICS}$ of pre-pubertal children with young adult males and females, using the PRBS technique. The indices of aerobic performance of the three groups were established using traditional methods of measuring $\dot{V}O_{2\,MAX}$, T_{vent} and the oxygen cost of cycling. The rationale for using the PRBS technique, frequency domain analysis and the selection of the study populations

is detailed below.

5.1.1. Rationale for the Frequency Domain Analysis of Oxygen Uptake Kinetics in Children

Intermittent activity characterises the exercise patterns of children (Cooper, 1995) and the measurement of $\dot{V}O_{2\ KINETICS}$ in the frequency domain may provide a more appropriate measure of the child's acute response to exercise as the exercise stimuli reflects the responses elicited in everyday life.

All previous comparisons of $\dot{V}O_{2\ KINETICS}$ between adults and children have been made in the time domain. The dominant mechanisms controlling $\dot{V}O_{2\ KINETICS}$ may be different in children compared to normal adults and need not necessarily be due to one component. Most of the models used to describe the response have been derived using adult data and have not been evaluated for use in the measurement of $\dot{V}O_{2\ KINETICS}$ in children. Therefore, at the present stage of knowledge it seems desirable to reduce the amount of explicit modelling used in characterising the $\dot{V}O_{2\ KINETICS}$ of children rather than to try and improve the models.

Another potential limitation of the time domain approach employed in previous studies is that the exercise tests have not been repeated as often as recommended (Lamarra et al., 1987), presumably because of time constraints or the supposition that children would become bored or were unable to perform too many repeats. In some studies (Cooper et al., 1985; Zanconato et al 1991; Hebestreit et al., 1998) tests involving children have been carried out over a period of two or more days. As the effects of diurnal variation, temperature, previous activity and diet on oxygen uptake kinetics is largely unknown, it is possible that variation in these factors may have compromised the interpretation of the results.

Frequency domain tests have obvious advantages, one of which is that the test procedure can be concluded in a period of 30 minutes or less. The need to fit an explicit model to characterise the data is not required, and the issue of scaling the physiological response to account for the size of the individual is not a consideration. The PRBS protocol provides a simple sub-maximal exercise test that has been shown to be well

tolerated by children (Kusenbach et al., 1999) and provides reliable information on $\dot{V}O_{2\,KINETICS}$ (Hoffman et al.,1994b; Hughson et al.,1990a; Kusenbach et al. 1994). A summary of the rationale for frequency domain analysis is provided in Table 5.1.

Table 5.1. Summary of the rationale for frequency domain analysis

- Responses are confined to simple oxygen uptake to work rate ratios negating the use of any scaling techniques.
- No explicit modelling is required.
- Analysis of the response to the PRBS protocol integrates a significant component of noise reduction (c.f. Section 3.3.2 below). The higher frequency responses associated with noise or the Phase I component are reduced or eliminated.
- The sub-anaerobic threshold work rate transitions mean that motivational requirements are low and the process may be suitable for clinical studies.
- The subject does not need to achieve exercise targets in order to accept the test as valid (c.f. $\dot{V}O_{2\,MAX}$).
- Multiple tests on several days are not required.
- As the responses represent a series of sinusoids oscillating about a mean level an expression of dynamic work economy can easily be derived from $\Delta WR / \Delta \dot{V}O_2$.
- The oxygen uptake response to appropriately constructed PRBS protocols below the anaerobic threshold have been shown to be dynamically linear in adults, therefore are appropriate stimuli for investigating $\dot{V}O_{2\,KINETICS}$.

5.1.2. Rationale for the Use of Noise Reduction Techniques in the Measurement of Oxygen Uptake Kinetics in Children

One potential problem inherent in the measurement of $\dot{V}O_2$ responses to sub-maximal work rate transitions in children lies in the small work rate changes that can be employed. This means that there will be less differentiation between the upper and lower $\dot{V}O_2$ responses with a resultant consequence of a larger potential influence of signal noise. Before effective comparisons between children and adults can be carried out it is important that methodological issues of noise reduction are addressed. The study is designed to include the calculation of an effective lung volume to minimise breath-to-breath variability and the application of auto– and cross-correlation techniques to significantly improve the signal to noise ratio.

5.1.3. Rationale for the Selection of Study Groups

It is necessary to design an investigation which removes the compounding influences of

ageing and relative fitness. Some of the previous studies which compare the $\dot{V}O_{2\,KINETICS}$ of children with adults, indices such as aerobic capacity and anaerobic threshold or other factors relating to the physical characteristics of the cohort such as the oxygen cost of cycling, have not been addressed in the study design or analysis. It is therefore important that this information is reported so that the groups can be clearly defined. The groups selected must also represent normal, healthy active adults and children, who have not engaged in a formal fitness training programs. This will improve the interpretation of the results as the need to partition out the effects that training on $\dot{V}O_{2\,KINETICS}$ is removed. Similarly, due to the gender differences normally associated with physical performance of adults it is necessary to compare the $\dot{V}O_{2\,KINETICS}$ of adult males and females independently with the $\dot{V}O_{2\,KINETICS}$ of children. This approach follows the study design of Turley and Wilmore (1997).

The age range of the three cohorts is also important in that the adults need to be mature but in early adulthood in order to negate any potential influence of the ageing process on $\dot{V}O_{2\,KINETICS}$. The children should be pre-pubertal as it is unlikely that gender effects will have manifested themselves at this stage of development.

5.2. The Aims of the Study

The aims of the study were to:

- design an appropriate methodology to measure the Phase II $\dot{V}O_{2\,KINETICS}$ in children,
- apply the technique to identify possible child-adult differences in Phase II $\dot{V}O_{2\,KINETICS}$.

5.3. Methods

5.3.1. Subjects

Ten children (7 males and 3 females) and 20 young adults (10 males and 10 females) agreed to participate in this study.

5.3.1.1. Children

The children were drawn from a group of 20 children who had been selected by age and sex as control subjects for a previously published evaluation (Marven et al., 1998 Appendix 5.1). The 10 children were chosen on the basis of being pre- and circum-pubertal. Using an interview to determine level ofparticipation in physical activity, all the children were established as being physically active with half of the subjects taking part in club activities as well as school sports. None of the children were engaged in a formal training programme.

All children were above the minimum height for testing which was 1.25 m and had reached Tanner stages 1, 2 or 3 (c.f. Chapter 4.1.4) as shown in the Table 5.2.

Table 5.2. Maturation stage (Tanner, 1962) of the 10 children.

Tanner stage	Stage 1	Stage 2	Stage 3
Males (n)	6	1	0
Females (n)	1	1	1

The physical characteristics of the children are shown in Table 5.3.

Ethics approval for the study was gained from the South Sheffield Ethics Committee and at all stages of exercise testing a paediatrician was present.

Informed consent was obtained from the children and their parent/guardian (Appendix 5.2). A parent / guardian or their representative was present at all stages of exercise testing.

5.3.1.2. Adults

Twenty healthy, young adults were drawn from the University student population. Using an interview to determine level of habitual physical activity, all the adults were established as being physically active individuals who participated in exercise on a regular basis, but were not engaged in any formal training programme. Approval for the study was given by the Sheffield Hallam University Research Degrees Committee.

Prior to inclusion in this study all subjects were screened for cardiovascular, respiratory

and musculo-skeletal disorders (Appendix 5.3). Written informed consent was obtained from each individual.

The physical characteristics of all subjects are shown in Table 5.3.

Table 5.3. Physical characteristics of children, adult females and adult males. Mean values (S.D.).

Group	Age (years)	Height (m)	Weight (kg)
Children (n=10)	10.81 (1.46)	143.3*** (8.1)	35.9*** (7.2)
Adult males (n=10)	23.79 (2.23)	179.7§§§ (7.1)	79.8§§§ (8.3)
Adult females (n=10)	21.62 (1.52)	164.3††† (5.8)	64.2††† (7.2)

*** $P < 0.001$ Children significantly different from adult males
§§§ $P < 0.001$ Adult males significantly different from adult females
††† $P < 0.001$ Adult females significantly different from children

5.3.2. Exercise testing

All subjects performed two tests. The initial test was a pseudo-random binary sequence (PRBS) exercise test for the determination $\dot{V}O_{2\ KINETICS}$ in the frequency domain (c.f. Chapter 4, Section 4.2.2.). This test was followed by a progressive exercise test to exhaustion using an incremental ramp protocol for the determination of maximum aerobic capacity ($\dot{V}O_{2\ MAX}$) ventilatory threshold (T_{vent}), oxygen cost and Δefficiency of cycling (c.f. Chapter 4, Sections 4.2.1. and 4.2.3.).

5.3.3. Data Analysis

Prior to any analysis unusual (non-physiologic) $\dot{V}O_2$ data, identified visually as outliers, were removed from the breath-by-breath data record.

5.3.3.1. Progressive Exercise Test

The breath-by-breath data were used to calculate 30 second averages for $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, heart rate and RER.

Confirmation that the subject had attained maximum aerobic capacity was determined by establishing the criteria for a plateau in oxygen uptake with respect to increasing

work rate, or by considering the additional criteria described in Chapter 4, Section 4.2.1.1 and Figure 4.9.

5.3.3.1.1. Ventilatory Threshold

Ventilatory threshold was identified as the $\dot{V}O_2$ which corresponds to the point when $\dot{V}E$ initially increased out of proportion with $\dot{V}O_2$ and was located using the method described by Whipp et al. (1981) described in Chapter 4, Section 4.2.1.2.

5.3.3.2. Pseudo-random Binary Sequence Exercise Test

After calculation of the effective lung volume (Chapter 4, Section 4.1.2.5), breath-by-breath oxygen uptake data was linearly interpolated and Fourier analysis was performed on the auto- and cross-correlated input and output respectively (Chapter 4, Section 4.1.2.6 and Figure 4.7.). The relationship between the ergometer input and $\dot{V}O_2$ output was calculated as an amplitude ratio ($ml \cdot min \cdot W^{-1}$) and phase delay (degrees) for the harmonic frequencies 2.2, 4.4, 6.7 and 8.9 mHz (Chapter 4, Section 4.2.2.1).

5.3.3.3. Delta Efficiency and Oxygen Cost of Cycling

The Δefficiency and oxygen cost of cycling was calculated using both the progressive exercise test and the PRBS test data sets (c.f. Chapter 4 Section 4.2.3).

From the progressive exercise test, oxygen cost of cycling ($ml \cdot min^{-1} \cdot W^{-1}$) was calculated using regression analysis and Δefficiency (%) estimated using the equation :

$$\Delta\text{efficiency (\%)} = \frac{291}{\text{gradient}}$$

The mean $\dot{V}O_2$ to work rate response was also calculated (Harmonic 0 from the Fourier analysis) in order to assess the overall oxygen cost of cycling during the PRBS exercise.

5.3.4. Statistical Analysis

Differences in the $\dot{V}O_{2\,KINETICS}$ between the three groups of subjects and between the amplitude or phase shift variables, were assessed using a two way analysis of variance (ANOVA). A one way ANOVA was used to analyse data where only single measurements were made. A post-hoc Tukey test was used to locate differences

between means. Statistical significance was set at $P < 0.05$.

5.4. Results

Results are expressed as means (S.D.) unless otherwise stated.

5.4.1. Progressive Exercise Test

5.4.1.1 Maximal Responses

The duration of the incremental ramp test for each group were 9.36 (± 2.2) minutes (children) 12.75 (± 1.47) minutes (adult males) and 11.77 (± 0.77) minutes (adult females). A plateau for $\dot{V}O_2$ was observed in 20% of children, 50% of adult males and 10% of adult females. Work rate, heart rate and respiratory responses at exhaustion are shown in Table 5.4.

Table 5.4. Cardio-respiratory variables measured at termination of the progressive exercise test.

	Work rate‡ (W)	Heart rate (beats·min^{-1})	$\dot{V}O_{2\,MAX}$ (ml·min^{-1})	$\dot{V}O_{2\,MAX}$ (ml·kg^{-1}·min^{-1})	RER	VE (l·min^{-1})
Children (n=10)	139*** (32)	185 (11)	1606*** (391)	44.7 (4.7)	1.12** (0.05)	57*** (11)
Adult Males (n=10)	343§§§ (36)	185 (11)	3390§§§ (437)	42.5§§ (3.6)	1.19 (0.04)	142§§§ (18)
Adult Females (n=10)	218††† (16)	187 (10)	2353††† (184)	36.9††† (2.9)	1.20††† (0.04)	81††† (16)

*** $P < 0.001$, ** $P < 0.01$, Children significantly different from adult males
§§§ $P < 0.001$, §§ $P < 0.01$ Adult males significantly different from adult females
††† $P < 0.001$ Adult females significantly different from children

All variables shown in Table 5.4 were calculated as the highest 30 s average values with the exception of work rate which is the highest work rate attained prior to termination of the test.

5.4.1.2. Ventilatory Threshold

In absolute terms (ml·min^{-1}) adult males had significantly higher ventilatory thresholds ($P < 0.001$) compared to children and adult females. Adult females also had significantly higher ventilatory thresholds than the children ($P < 0.01$). When expressed

relative to body mass ($ml \cdot kg^{-1} \cdot min^{-1}$) there was no significant difference in T_{vent} between the adult groups, but adults females had significantly lower ventilatory threshold's compared to children ($P < 0.01$). Ventilatory threshold was not significantly different between the three groups when expressed as a percentage of $\dot{V}O_{2\,MAX}$ as shown in Table 5.5.

Table 5.5. Ventilatory threshold expressed in absolute terms, relative to body mass and as a percentage of maximum oxygen uptake.

	T_{vent} ($ml \cdot min^{-1}$)	T_{vent} ($ml \cdot kg^{-1} \cdot min^{-1}$)	T_{vent} ($\%\dot{V}O_{2\,MAX}$)
Children (n=10)	1115*** (285)	31.0 (3.7)	69.6 (5.4)
Adult Males (n=10)	2248§§§ (439)	28.2 (4.4)	66.2 (6.8)
Adult Females (n=10)	1590†† (159)	25.0†† (3.0)	67.6 (5.0)

***P < 0.001 Children significantly different from adult males
§§§ P < 0.001 Adult males significantly different from adult females
†† P < 0.01 Adult females significantly different from children

5.4.2. Pseudo-random Binary Sequence Exercise Test

5.4.2.1. Work Intensity

The highest 30 s value for $\dot{V}O_2$ achieved during the PRBS exercise was calculated as a percentage of T_{vent}. For the children this represented a relative intensity of 89.0% T_{vent}, for the adult males 63.5% T_{vent} and, for the adult females, 77.5% T_{vent}.

5.4.2.2 Oxygen Uptake Kinetics : Amplitude Ratio

Two way ANOVA showed the expected significant decreases in amplitude ratio as the frequency increased from 2.2 - 8.9 mHz ($P < 0.001$), and identified differences between the groups of children and adults ($P < 0.001$).

The amplitude ratios expressed as $ml \cdot min^{-1} \cdot W^{-1}$ are shown in Table 5.6.

When the four harmonics for each group are pooled, post hoc Tukey analysis, showed that children had significantly higher amplitude ratios when compared to either of the adult groups ($P < 0.001$). In addition, the adult females had significantly higher

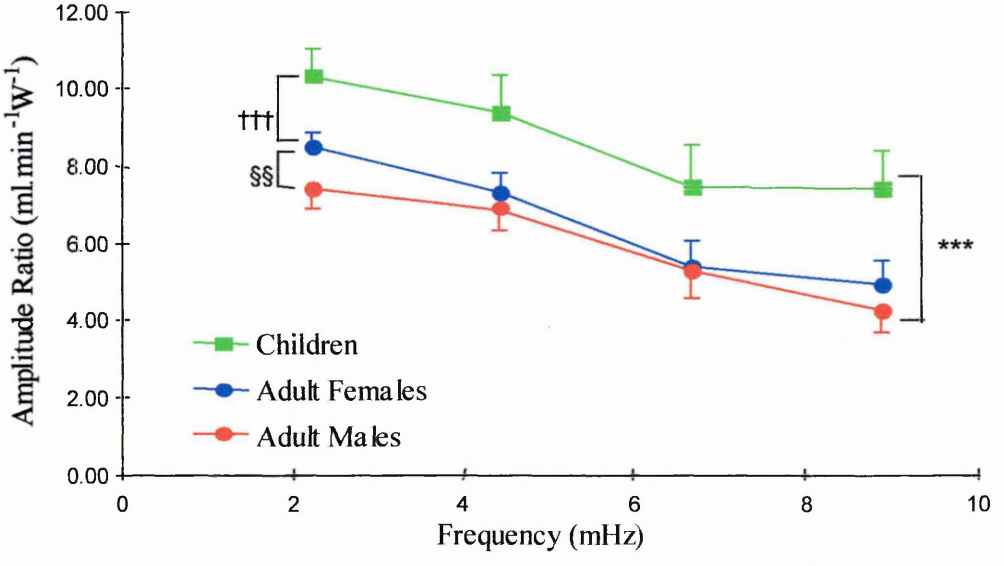
amplitude ratios when compared with those of the adult males ($P < 0.01$).

Table 5.6. Oxygen uptake kinetic responses (amplitude ratio $ml \cdot min^{-1} \cdot W^{-1}$) of children, adult males and adult females.

	Frequency (mHz)			
	2.2	4.4	6.7	8.9
	Amplitude Ratio ($ml \cdot min^{-1} \cdot W^{-1}$)			
Children (n=10) ***	10.33*** (0.73)	9.42*** (0.99)	7.46*** (1.14)	7.42*** (0.99)
Adult Males (n=10) §§	7.43§ (0.50)	6.91 (0.58)	5.32 (0.72)	4.25 (0.54)
Adult Females (n=10) †††	8.52††† (0.34)	7.32††† (0.51)	5.41††† (0.70)	4.96††† (0.57)

*** $P < 0.001$ Children significantly different from adult males
§§ $P < 0.01$, §$P < 0.05$ Adult males significantly different from adult females
††† $P < 0.001$ Adult females significantly different from children

The post-hoc Tukey analysis used to locate differences between individual means indicates that, at each of the 4 harmonics, children have significantly higher amplitude ratios ($P < 0.001$) than either of the two adult groups. At harmonic 1 (2.2 mHz) adult females have a significantly higher amplitude ratio than adult males ($P < 0.05$).



*** $P < 0.001$ Children significantly different from adult males
§§ $P < 0.01$ Adult males significantly different from adult females
††† $P < 0.001$ Adult females significantly different from children

Figure 5.1. Amplitude ratio of the children, adult males and adult females.

The results from all groups showing the relationship between amplitude ratio and frequency are presented as a Bode plot Figure 5.1.

5.4.2.3. Oxygen Uptake Kinetics : Phase Delay

Two way analysis of variance showed the expected significant increases in phase delay as the frequency increased from 2.2 - 8.9 mHz ($P < 0.001$), and identified differences between the groups of children and adults ($P < 0.001$).

Over the 4 harmonics, post hoc analysis between subject groups, showed that children had significantly smaller phase delays compared to either adult males ($P< 0.01$) or adult females ($P < 0.001$). There was no significant difference in phase delay between the adult males and females.

The phase delays of the children, adult males and adult females are shown in Table 5.7.

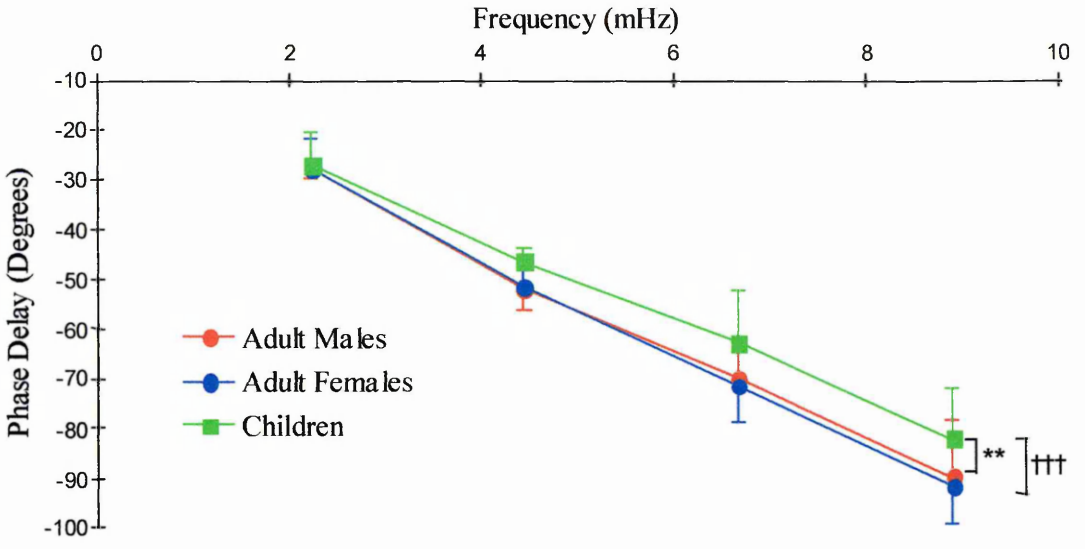
Table 5.7. Oxygen uptake kinetic responses (phase delay in degrees) of children, adult males and adult females. (negative values express delay in relation to work rate change).

	Frequency (mHz)			
	2.2	4.4	6.7	8.9
	Phase Delay (Degrees)			
Children (n=10) **	-26.78 (6.37)	-46.22 (2.49)	-62.67 (10.60)	-81.93 (10.45)
Adult Males (n=10)	-27.54 (6.01)	-51.74 (3.74)	-69.65 (6.50)	-89.53 (11.63)
Adult Females (n=10) †††	27.66 (1.86)	-51.29 (4.82)	-71.18 (7.71)	-91.69 (7.24)

** $P < 0.01$ Children significantly different from adult males
††† $P < 0.001$ Adult females significantly different from children

The post hoc Tukey analysis did not identify any significant differences between groups when individual means (phase delay) at each harmonic were compared (Table 5.7).

The results from all groups showing the relationship between phase delay and frequency are presented as a Bode plot Figure 5.2



** P < 0.01 Children significantly different from adult males (all Harmonics)
††† P < 0.001 Adult females significantly different from children (all Harmonics)

Figure 5.2. Phase delay of the children, adult males and adult females.

5.4.3. Delta Efficiency and Oxygen Cost of Cycling

Delta efficiency and oxygen cost of cycling were calculated using measurements made during both the ramp and the PRBS exercise tests (Table 5.8).

Table 5.8. Delta efficiency and oxygen cost of cycling during the ramp and the PRBS exercise tests.

	Δefficiency (ramp) (%)	Oxygen cost (ramp) ($ml \cdot min^{-1} \cdot W^{-1}$)	Oxygen cost‡ (PRBS) ($ml \cdot min^{-1} \cdot W^{-1}$)
Children (n=10)	26 * (3)	11.3* (1.5)	23.4*** (1.5)
Adult Males (n=10)	31 (5)	9.4 (1.4)	20.7 (1.2)
Adult Females (n=10)	34†† (4)	8.7††† (1.0)	19.7††† (1.3)

*P < 0.05, ***P < 0.001 Children significantly different from adult males
††P < 0.01, †††P < 0.001 Adult females significantly different from children

‡The value for oxygen cost during the PRBS protocol is the mean $\dot{V}O_2$ / work rate relationship (Amplitude ratio of Harmonic 0) and unlike the value for oxygen cost during the ramp protocol it does not solely relate to the oxygen cost of work but includes the resting metabolism oxygen requirement.

In terms of oxygen cost of cycling, children require significantly more oxygen per unit of work than do adult males ($P < 0.05$) and adult females (0.001) when performing incremental aerobic exercise. When expressed in terms of Δefficiency, children were shown to be significantly less efficient when performing aerobic work during the ramp protocol than adult males ($P < 0.05$) and adult females ($P < 0.01$). This is also reflected in the results for the oxygen cost of cycling calculated during the PRBS exercise test; children required significantly more oxygen per unit of work than do either of the adult groups ($P < 0.001$).

5.5. Discussion

5.5.1. Study Population

Any study which aims to examine the differences in the physiological responses to exercise in children and adults needs to carefully consider the choice of study population. The children need to be relatively young (Turley and Wilmore, 1997) and the pubertal stage identified. The adults must be mature but not have started the ageing process (Babcock et al., 1994a; Chillibeck et al., 1996). It was therefore important that the children were examined by a qualified auxologist from The Sheffield Children's Hospital in order to accurately identify the stage of sexual development. The age range of the children was 8.4 to 13.0 years and seven of the children were identified as being pre-pubertal, the remaining children were Tanner stage 3 or below. The adults were of age range 22 – 28.4 years (males) and 19.6 – 24.9 years (females).

5.5.2. Cardio-respiratory Fitness

Evidence suggests that the $\dot{V}O_{2\,KINETICS}$ are similar in older individuals who are active when compared to a younger, sedentary population (Chillibeck et al., 1996). It is therefore important that any difference in cardiovascular fitness between the 3 groups used in this study is accounted for. One method of accounting for any fitness related differences in $\dot{V}O_{2\,KINETICS}$ is to employ statistical modelling techniques (Chillibeck et al., 1996). An alternative approach was adopted in the current investigation was to select the groups on the basis of them having similar activity profiles. Although it was

not possible to precisely determine the activity levels of the three groups it was possible, through an interview technique, to identify that all subjects considered themselves active and none were undertaking formal physical training.

To provide a quantitative description of the aerobic performance characteristics the parameters of $\dot{V}O_{2\ MAX}$ and T_{vent} were determined.

The range in recorded $\dot{V}O_{2\ MAX}$ for the adult males was 36.2 to 47.5 $ml \cdot kg^{-1} \cdot min^{-1}$, for adult females 30.9 to 40.2 $ml \cdot kg^{-1} \cdot min^{-1}$ and for children 37.0 to 54.3 $ml \cdot kg^{-1} \cdot min^{-1}$. For the children, average values of 44.7 (± 4.7) $ml \cdot kg^{-1} \cdot min^{-1}$, compared favourably with the results of Mahon et al. (1997) which were 47.4 (± 5.4) $ml \cdot kg^{-1} \cdot min^{-1}$ in normal children of similar age. Armstrong et al. (1991) reported $\dot{V}O_{2\ PEAK}$ values of 43 (± 7) and 37 (± 6) $ml \cdot kg^{-1} \cdot min^{-1}$ in boys and girls of age range ~11 to 15 years, respectively. For the adult males the average value for $\dot{V}O_{2\ MAX}$ was 42.5 (± 3.6) $ml \cdot kg^{-1} \cdot min^{-1}$ which falls between the levels reported for untrained males (Saltin and Astrand, 1967) and those reported for college students (McArdle et al., 1991) performing maximal cycle ergometer tests. The average aerobic power of the adult females (36.9 ± 2.9 $ml \cdot kg^{-1} \cdot min^{-1}$) was identical to the values reported by Cunningham et al. (1993) and also comparable to those reported by Pollock et al. (1982) for healthy, sedentary, young women performing cycle ergometer exercise (33.3 ± 5.2 $ml \cdot kg^{-1} \cdot min^{-1}$). The finding that the adult females had significantly lower $\dot{V}O_{2\ MAX}$ in relation to body weight than either the adult males or the children is supported in the study of Turley and Wilmore (1997).

The $\dot{V}O_{2\ MAX}$ values recorded in this study are considerably lower than those reported for trained individuals. Trained children aged 10.8 years achieved average $\dot{V}O_{2\ MAX}$ values of 58.6 ± 3.9 $ml \cdot kg^{-1} \cdot min^{-1}$ during treadmill exercise (Rotstein et al., 1986). Treadmill measures of $\dot{V}O_{2\ MAX}$ are always higher than those attained during cycling. In a study of young competitive swimmers an average $\dot{V}O_{2\ MAX}$ of 56.8 $ml \cdot kg\text{-}^{1} \cdot min^{-1}$ was recorded during cycling exercise. Similarly, the adult values are lower than would be expected from trained individuals (Bunc and Heller, 1993; Saltin and Astrand, 1967) The $\dot{V}O_{2\ MAX}$ values recorded for the three groups used in this study can therefore be considered normal for untrained individuals.

A comparison of the T_{vent} values measured in terms of both $ml \cdot kg^{-1} \cdot min^{-1}$ and as $\%\dot{V}O_{2\,MAX}$ also supports the fact that the children are representative of a normal population (Mahon et al., 1997) rather than a trained population (Bunc and Heller, 1993). The ventilatory thresholds were very similar for all groups when expressed as $\%\dot{V}O_{2\,MAX}$ and $ml \cdot kg^{-1} \cdot min^{-1}$.

This evidence supports the selection procedure for the study groups and it may be concluded that factors relating to cardio-respiratory fitness of the three groups are unlikely to have had a significant effect on the interpretation of the $\dot{V}O_{2\,KINETICS}$ results.

5.5.3. Oxygen Cost of Exercise

In spite of the powerful argument of Rowland et al. (1990) that there are no child-adult differences in the mechanical efficiency of work performed on a cycle ergometer, the results from this study were unable to confirm these findings. Both the adult males ($P < 0.05$) and adult females ($P < 0.01$) in the current study had significantly higher Δefficiencies than the children. This finding is unlikely to have been biased by differences in substrate utilisation, as estimated from RER, because the analysis of Δefficiency was constrained to moderate intensity exercise between the second minute of the ramp protocol and 90% of T_{VENT}. Respiratory exchange ratios over this range of intensities fell between values of 0.87 and 0.93 and were not significantly different between the three groups. The findings from the current study are however supported by the work of Turley and Wilmore (1997) who reported mechanical efficiencies of 35.4% for adult males, 31.5% for adult women and 25.1% for the children. These values are very similar to the Δefficiencies values of 31.5% (adult males), 34.0 % (adult females) and 26.4% (children) recorded in the current study. The average Δefficiency results for the children in this study are also similar to the average mechanical efficiency of 23.2% determined in boys by Rowland et al. (1990). The fact that Rowland et al. (1990) found no child-adult differences may be explained by the poor mechanical efficiency of 22.5% demonstrated by their adult males.

The higher oxygen cost of the children (11.3 ± 1.5 $ml \cdot min^{-1} \cdot W^{-1}$) compared to adult

males (9.4 ± 1.4 $ml \cdot min^{-1} \cdot W^{-1}$) and adult females (8.7 ± 1 $ml \cdot min^{-1} \cdot W^{-1}$) during the PRBS test also supports the finding that children use more oxygen to deliver the same work output. For the children to achieve the same power output as the adults, it is possible that the children use a smaller absolute amount of muscle mass (Katsuura, 1986). This would account for the higher a-$\bar{v}O_2$ difference in children during sub-maximal exercise (Turley and Wilmore, 1997). The effects of using a smaller muscle mass would be the generation of a larger amounts of metabolic bi-products (Lewis et al., 1985) and heat per unit of muscle. This would have the effect of increasing oxyhaemoglobin dissociation, increasing muscle blood flow and increasing heart rate through metabolic reflex control. These mechanisms may explain why the oxygen cost of cycling is higher in children since there is no evidence to support poorer cycling mechanics in children compared to adults.

An additional explanation offered for the lower mechanical efficiency of children compared to adults observed in the present study not found in the study of Rowland et al. (1990) may be associated with the relative intensity of the work rate used. The pre-pubertal subjects in the Rowland et al. (1990) investigation were cycling at almost twice the relative intensity of the adults at a work load of 45 W. The use of the ramp protocol in the present study, and only calculating Δefficiency using data up to 90% T_{VENT} means that the same relative intensities were used in the calculation of Δefficiency for the three groups. An additional consideration may also be related to the significantly depressed efficiency at low work loads (Klausen et al., 1985; Rowland et al. 1990) which has been attributed to the increased energy necessary to move the legs, fix the trunk, and overcome the inertial friction of the ergometer. This phenomenon may have been an influencing factor affecting the results of this study as relatively low intensities were used both in the initial stages of the ramp protocol and during the PRBS test.

It is concluded that there was a higher oxygen cost of cycling at sub-maximal work intensities in children compared to adults. There are several possible mechanisms which may be responsible for this difference including the unexplained factors relating to relative exercise intensity. This may account for the opposing observations of other

researchers (Rowland et al., 1990).

5.5.4. The Measurement of Oxygen Uptake Kinetics

5.5.4.1. Comparison with Published Data

The PRBS sequence used in this investigation was identical to that described by Eßfeld et al. (1987) in a study of a mixed cohort of 10 male and female students whose average $\dot{V}O_{2\,MAX}$ was less than 50 $ml \cdot kg^{-1} \cdot min^{-1}$. The amplitude ratio and phase delay results of the adult females and adult males of this study compare favourably with the values reported by Eßfeld et al. (1987) as shown in Figures 5.3 (a) and (b).

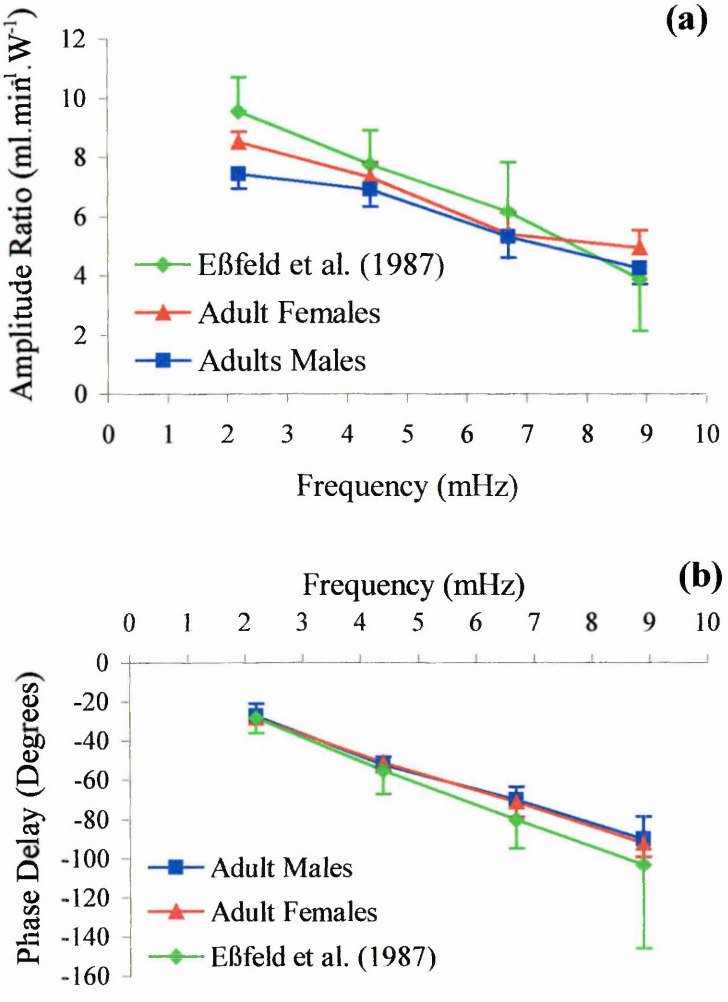


Figure 5.3. A comparison between the amplitude ratio (a) and phase delay (b) of adult males (n=10) and adult females (n=10) with a mixed sex cohort (n=10) of adults with aerobic capacities $< 50\ ml \cdot kg^{-1} \cdot min^{-1}$ (Eßfeld et al. 1987).

In contrast to the $\dot{V}O_{2\ KINETICS}$ of the adults in the present study which were similar in terms of both amplitude ratio and phase delay to subjects with aerobic capacities of < 50 $ml \cdot kg^{-1} \cdot min^{-1}$, the children compared more favourably with the $\dot{V}O_{2\ KINETICS}$ of the adult subject groups with high aerobic capacities Figure 5.4 (a) and (b).

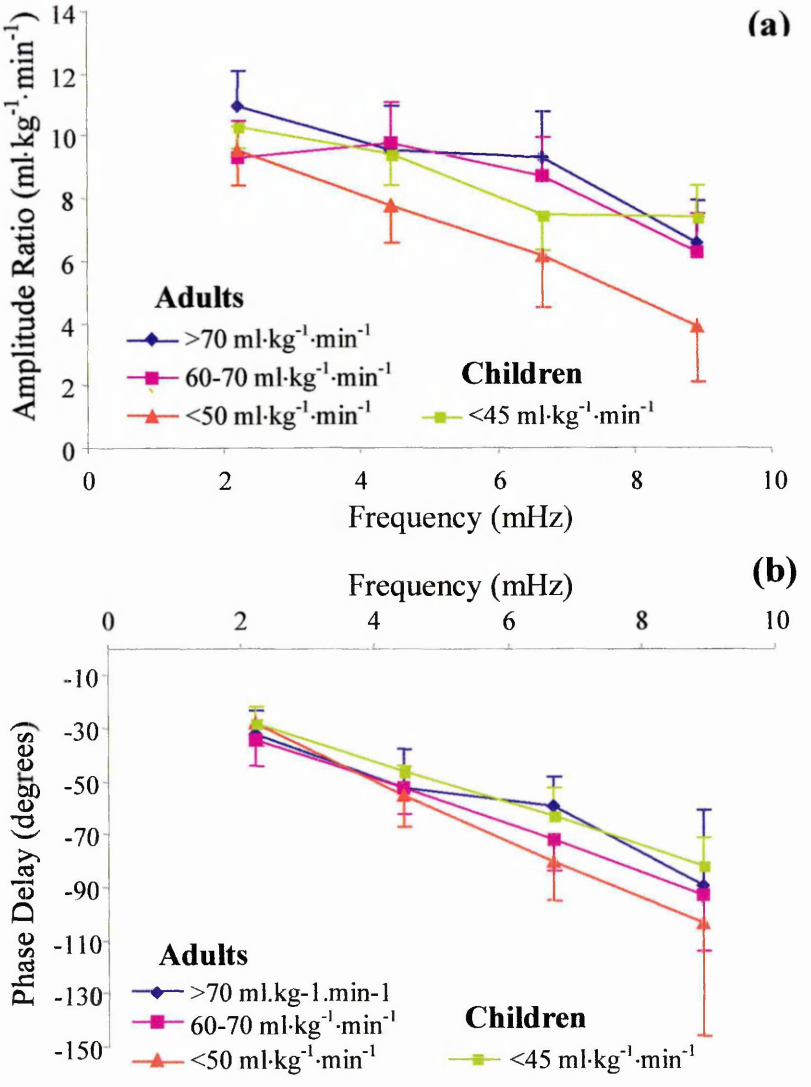


Figure 5.4. A comparison between the amplitude ratio (a) and phase delay (b) of children (n=10) with a mixed sex cohort of adults of different aerobic capacities reported by Eßfeld et al., 1987)

5.5.4.2. Comparisons between Children and Adults

The findings of this study are in opposition to the results of Springer et al. (1991) and Hebestreit et al. (1998) who recorded similar Phase II $\dot{V}O_{2\ KINETICS}$ in adults and children and concluded that the factors limiting cellular respiration (Phase II

$\dot{V}O_{2\,KINETICS}$) are constant throughout growth. In making these observations, Hebestreit et al. (1998) had compared the below ventilatory threshold, Phase II $\dot{V}O_{2\,KINETICS}$ of nine boys of age range 9 to 12 years with eight men aged between 19 and 27 years, using a step transition in work rate which was repeated once. The test commenced with unloaded cycling for two minutes followed by 3.5 minutes of exercise at a work rate equivalent to 50% $\dot{V}O_{2\,PEAK}$. The cadence of 80 revolutions per minute was high for sub-maximal exercise, but this was necessary in order to perform the higher work intensities (up to 135% $\dot{V}O_{2\,PEAK}$) which were also used in the Hebestreit et al. (1998) study. The results showed that the oxygen deficits (calculated for the whole response) were greater in the men (387 ml O_2) compared to boys (219 ml O_2), but this difference was eliminated when the results were expressed relative to body weight. The time constant (τ) for Phase II $\dot{V}O_{2\,KINETICS}$ was the same in boys (22.79 ± 5.05 s) and men (26.4 ± 4.1 s) in spite of a significantly greater oxygen cost for the boys (10.9 ± 2.2 $ml \cdot min^{-1} \cdot W^{-1}$) compared to the men (9.7 ± 0.9 $ml \cdot min^{-1} \cdot W^{-1}$). Hebestreit et al. (1998) results therefore challenge the common belief that children rely less on anaerobic energy turnover early in exercise.

If the choice of subject groups is taken into account in the Hebestreit et al. (1998) study it may however allow us to consider an alternative explanation to that drawn above. The adult volunteers, although they were not elite athletes, had higher aerobic capacities (53.4 (± 7.4) $ml \cdot kg^{-1} \cdot min^{-1}$) than the children (47.3 (± 5.5) $ml \cdot kg^{-1} \cdot min^{-1}$). As subjects with higher aerobic capacities have consistently been reported to have improved $\dot{V}O_{2\,KINETICS}$ (Whipp and Wasserman, 1972; Hickson, et al., 1978; Hagberg et al., 1978; Powers et al., 1985; Eßfeld et al., 1987; Zhang et al., 1991) this factor alone could have resulted in the adult males in the study of Hebestreit et al. (1998) having faster $\dot{V}O_{2\,KINETICS}$ than might have been expected, thereby obscuring any potential child-adult differences due solely to maturational differences.

In the study of Springer et al. (1991) Phase II $\dot{V}O_{2\,KINETICS}$ were compared in a group of 9 children (5 boys) of mean age 8.2 ± 1.4 years, with a group of nine adults (5 males) of age range 18 to 40 years. The τ $\dot{V}O_2$ were reported to be similar in children (τ = 23.9 ±

4.6 s) to adults (26.8 ± 4.3 s). It is difficult to interpret fully the conclusions drawn by Springer et al. (1991) because the group of adults who performed the $\dot{V}O_{2\ KINETICS}$ test did not undertake a test of $\dot{V}O_{2\ MAX}$ or T_{vent}. It is therefore impossible to determine the intensity used during the $\dot{V}O_{2\ KINETICS}$ test nor is it possible to speculate on the effects that the aerobic capacity of the volunteers may have had on the results. Of the other studies which have found differences in the $\dot{V}O_{2\ KINETICS}$ of children and adults (Macek and Vavra, 1980a and b; Sady, 1981; Freedson et al., 1981; Macek et al., 1984 and Armon et al., 1991) all have measured $\dot{V}O_{2\ KINETICS}$ in the time domain and have either employed inappropriate modelling techniques and/or have not accounted for the three phases of the oxygen uptake response. There is therefore only limited empirical evidence available regarding the developmental differences in $\dot{V}O_{2\ KINETICS}$ between adults and children which incorporates the appropriate techniques for the analysis of Phase II $\dot{V}O_{2\ KINETICS}$.

By adopting the PRBS approach to the assessment of $\dot{V}O_{2\ KINETICS}$, the results of this study support the contention that $\dot{V}O_{2\ KINETICS}$ of pre- and circum-pubertal children are quantitatively different from a young adult population when the study populations are matched for activity and cardiovascular fitness. This finding is in opposition to the conclusions drawn by previous authors (Hebestreit et al., 1999; Springer et al. 1991), whose findings support the hypothesis of Cooper et al. (1985) that the rate of the $\dot{V}O_2$ response to exercise is influenced primarily by the need for cellular homeostasis and is age and size independent.

One possible explanation for the current observations may relate to the methodology itself. The analysis of the oxygen uptake response to the PRBS exercise discriminates between the amplitude and the phase delay as two interrelated, but separate, variables which are indicative of the dynamic relationship between work rate and oxygen uptake. This is not the case with the traditional approach of using a step transition to determine $\dot{V}O_{2\ KINETICS}$ employed in the studies of Hebestreit et al. (1998) and Springer et al. (1991). In the case of the step transition, the magnitude of the response is an estimated or measured steady state parameter that does not express the dynamic relationship

between work rate and oxygen uptake in the same way as the frequency domain analysis.

It could be argued that the step and the PRBS test modalities stimulate different physiological processes controlling the $\dot{V}O_2$ response. Hughson et al. (1991b), however, compared the $\dot{V}O_2$ response to both the step and the PRBS protocols and concluded that, in adults, the two exercise tests provide quantitatively similar results, that is, the dynamic linearity is preserved indicating that the responses to both the PRBS test and the step test are under the same physiological control i.e. permitting similar interpretation of the underlying control responses using both techniques.

A feature of the PRBS test is that, although Phase I kinetics have been shown to make only a relatively small contribution to the overall response in adults (Hughson et al., 1990a; Hoffmann et al. 1994b), it is not totally excluded from the analysis. By constraining the analysis to only the lower harmonics (periods greater than 100 s in the current study) the relative contribution of any high frequency responses (including Phase I) is minimised. It is unclear at the present time *above* which frequency the influence of the Phase I component may contribute significantly to the overall response. The influence of the Phase I component can therefore not be excluded as a contributory factor representing some of the differences observed in $\dot{V}O_{2\,KINETICS}$ between the adults and children in this study.

In the studies of Hebestreit et al. (1998) and Springer et al. (1991) it may be argued that the methodology employed provides inadequate confidence in the determined model parameter estimates of $\dot{V}O_{2\,KINETICS}$ as only single repetitions of the step response, or ensembled averaged responses from several groups of subjects performing a single transition, were analysed (c.f. Lamarra et al. 1987). In the current study the effects of breath-to-breath variability on the calculation of the frequency domain $\dot{V}O_{2\,KINETIC}$ parameters was reduced using two techniques. The initial approach was to use an estimate of alveolar gas exchange (Beaver et al. 1981), and a *post-hoc* calculation of an effective lung volume in order to minimise the breath-to-breath variation. This method has been shown to provide a good estimation of gas exchange at the alveolar level

(Swanson et al., 1981) and is recommended for use in studies of the kinetics of gas exchange (Beaver et al. 1981). Secondly, by adopting the use of the cross-correlation technique (which removes any $\dot{V}O_2$ responses not associated with the work rate stimuli) and constraining the analysis to only the lower frequencies, the influence of the inherent high frequency, breath-to-breath variability on the determination of $\dot{V}O_{2\,KINETICS}$ is reduced. This approach therefore treats the data in a fundamentally different way to the studies Hebestreit et al. (1998) and Springer et al. (1991), and may be a contributing factor in the opposing results obtained in this study.

The experimental methodology employed by Hebestreit et al. (1999) and Springer et al. (1991) uses discrete step protocols which removes the potential effect that prior exercise may have on $\dot{V}O_{2\,KINETICS}$. The nature of the PRBS test, however, means that the $\dot{V}O_{2\,KINETICS}$ have the potential to be influenced by the prior exercise transition. In an assessment of the effects of prior exercise on pulmonary gas exchange in adults Gerbino et al. (1996) have demonstrated that prior sub- or supra-threshold exercise does not affect the subsequent sub-threshold $\dot{V}O_{2\,KINETICS}$ in adults. There are currently no studies which have investigated this phenomenon in children and the potential influence of the previous exercise transition cannot therefore not be excluded as a contributory factor representing some of the differences observed in $\dot{V}O_{2\,KINETICS}$ between the adults and children in this study.

Some of the factors responsible for the two fold increase in 1-mile run performance between the ages of 5 and 15 years (Rowland, 1990) or the improvements in anaerobic power, which is independent of the increase in body dimensions (Rowland, 1995), are likely to indicate maturity associated changes in cardio-vascular function and the metabolic pathways essential for physical activity. Similarly, the differences in $\dot{V}O_{2\,KINETICS}$ observed between the adults and children in the current study may also indicate maturity associated differences in the processes controlling muscle oxygen utilisation. Theoretically it could be argued that in adults the control of $\dot{V}O_{2\,KINETICS}$ is driven by ATP demand in the skeletal muscle rather than the supply of oxygen to the muscle (Mahler, 1985; Yoshida and Whipp, 1994; McCreary et al. 1996; Meyer and

Foley, 1996). Experiments to determine the controlling factors in children have not been performed but since muscle mitochondrial capacity is higher in children than adults (Taylor et al., 1997) and oxidative enzymes are higher (Eriksson, 1972 and 1980; Haralambie, 1982; Berg et al., 1986), it is likely that the controlling factor relates to some aspect of peripheral metabolism. It is suggested that the findings of the current study support the contention that there are maturational differences between adults and children in the metabolic processes involved in the utilisation of oxygen during physical activity.

In conclusion, the sub-maximal nature of the cycle ergometer based PRBS test removes the problems associated with motivating the subjects to perform higher intensity tests such as is required in the tests to measure anaerobic threshold or maximum oxygen uptake. The results of the PRBS test are therefore not influenced by either the willingness or the ability of the subjects to sustain high intensity exercise. The analysis of the PRBS test does not require scaling to account for differences in body size which is an issue which requires addressing in most, if not all, other studies involving paediatric exercise. As the PRBS testing procedure is non-invasive and can be concluded in a single period of 20 minutes or less it offers a sensible additional approach to the testing of children's physiological responses to exercise.

The application of the PRBS method to the measurement of oxygen uptake responses of children and adults during exercise has demonstrated that the $\dot{V}O_{2\,KINETICS}$ of children, when measured in the frequency domain, are different to young adults. This effect can be quantified in terms of significantly higher amplitude ratios (across the frequency range 2.2 to 8.9 mHz) in children compared to young adults and significantly shorter phase delays (across the frequency range 2.2 to 8.9 mHz) in children compared to young adults. This effect is observed as an overall parameter and is not identifiable at any specific frequency.

$\dot{V}O_{2\,KINETICS}$ are known to be influenced by different training regimes in adults (Edwards et al., 1999). The PRBS technique could provide a useful ergonomic approach to assess the effectiveness of training in children without the difficulties associated with

partitioning out the influences of growth. The improvement in endurance performance of children during development cannot be explained by the traditional measurement of $\dot{V}O_{2\ MAX}$ (Rowland, 1990), therefore it would be interesting to speculate that measurements of $\dot{V}O_{2\ KINETICS}$ will provide a predictive measure of endurance performance throughout the period of maturation.

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Appendix 2.1

The generation of a 15 unit PRBS exercise protocol using a 4 unit shift register with modulo-2 adder feedback.

If (Stage 1 + stage 4) < 2 then normal arithmetic applies. If (Stage 1 + Stage 4) = 2 then the modulo-2 sum is given by Stage 1 + Stage 2 - 2

	Stage				1 = Low and 2 = High Work rate intensity
Unit Number	1	2	3	4	(Stage 1 + Stage 4) modulo-2 sum
1	1	0	0	0	1
2	1	1	0	0	1
3	1	1	1	0	1
4	1	1	1	1	0
5	0	1	1	1	1
6	1	0	1	1	0
7	0	1	0	1	1
8	1	0	1	0	1
9	1	1	0	1	0
10	0	1	1	0	0
11	0	0	1	1	1
12	1	0	0	1	0
13	0	1	0	0	0
14	0	0	1	0	0
15	0	0	0	1	1

Arrows denote : Shift all existing values one stage right into next row and insert feedback term from previous unit into stage 1.

After 15 iterations the sequence repeats from unit number 1 ad infinitum.

Appendix 2.2

Fourier program

```
10  REM Fourier analysis using Hughson (1990) definitions
20  REM ****************************************
100 DIM sig(1500), A(150), B(150), amp(150), phase(150)
200 INPUT "Type 1 for signal data from file, 0 for test data"; ff
210 INPUT "Type number of data points"; nmax
220 IF ff = 1 THEN
230     INPUT "Type filename...."; file$
240     OPEN file$ FOR INPUT AS #1
250     REM Here is the data input routine
260     REM nmax data points in array sig
270     REM *****************************
300     FOR i = 1 TO nmax
310             INPUT #1, ii
315             PRINT "ii =", ii
320     NEXT i
330     FOR i = 1 TO nmax
340             INPUT #1, sig(i)
345             PRINT "signal = ", sig(i)
350     NEXT i
355     CLOSE #1
360 ELSE
400     PRINT "Set up the "; nmax; "test data points"
410     FOR i = 1 TO nmax
415 REM                 CURRENTLY A SQUARE WAVE
420             IF i < nmax / 2 + .5 THEN
430                     sig(i) = 0
440             ELSE
450                     sig(i) = 1
460             END IF
470     NEXT i
480 END IF
500 REM ***************************
505 REM Now do the Fourier Analysis
510 REM ***************************
515 INPUT "Which number harmonic do you want to go to?"; maxh
520 PRINT "Doing the Fourier Analysis now....."
525 CONST pi = 3.14159
530 PRINT "frequency analysis results"
535 PRINT "No.", "An", "Bn", "Amp", "Phase"
540 FOR Q = 0 TO maxh
545     asum = 0
550     bsum = 0
555     FOR n = 1 TO nmax
560             theta = 2 * pi * Q * n / nmax
565             abit = COS(theta) * sig(n)
570             bbit = SIN(theta) * sig(n)
575             asum = asum + abit
580             bsum = bsum + bbit
585     NEXT n
586     REM Note what follows will give one half the usual formulae,
587     REM as Hughson's formulae do.
590     A(Q) = asum / nmax
591     B(Q) = bsum / nmax
592     amp(Q) = SQR(A(Q) * A(Q) + B(Q) * B(Q))
593     phase(Q) = ATN(B(Q) / A(Q)) * 180 / pi
```

```
594     PRINT USING "#.###"; Q;
595     PRINT USING "##########.###"; A(Q); B(Q); amp(Q); phase(Q)
596 NEXT Q
800     INPUT "Type 1 to save to file, 0 to end"; ff
801     IF ff = 1 THEN
802             INPUT "Type filename...."; file$
803             OPEN file$ FOR OUTPUT AS #2
810             FOR n = 0 TO maxh
811                     PRINT #2, A(n)
812             NEXT n
813             FOR n = 0 TO maxh
814                     PRINT #2, B(n)
815             NEXT n
816             FOR n = 0 TO maxh
820                     PRINT #2, amp(n)
830             NEXT n
840             FOR n = 0 TO maxh
850                     PRINT #2, phase(n)
860             NEXT n
891     END IF
940 END
```

Appendix 2.3

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RELIABILITY OF A PSEUDO RANDOM BINARY SEQUENCE TEST TO MEASURE OXYGEN UPTAKE KINETICS

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INTRODUCTION

In clinical and exercise physiology the assessment of oxygen uptake ($\dot{V}O_2$) kinetics can provide valuable information about functional capacity.

Various methods have been employed in the assessment of $\dot{V}O_2$ kinetics. One such method uses pseudo random binary sequence (PRBS) changes in work rate (Bennett 1981; Hughson 1990).

The PRBS technique provides a description of $\dot{V}O_2$ kinetics over a wide range of aerobic exercise intensities using a single test (Hoffmann 1994).

In a review of literature no evidence could be found of any studies that assessed the test / re-test reliability of the PRBS technique.

AIM

The aim of this investigation was to assess the test / re-test reliability of a specific PRBS test protocol by examining intra-subject variability in $\dot{V}O_2$ kinetics.

METHODS

Twenty healthy male subjects, age 26.2 ± 4.0 years (mean ± standard deviation (SD)), completed two identical PRBS protocols.

Each subject performed the tests on an electrically braked cycle ergometer.

The chosen protocol incorporated a series of six identical PRBS sequences. Each sequence consisted of 63 units of 5 s duration, giving a total period of 315 s. During each sequence the work rate was automatically alternated between 25 W and 105 W.

Respiratory gas exchange was measured on a breath-by-breath basis using a respiratory mass spectrometer. A III-lead electrocardiograph system was used to monitor heart rate.

DATA ANALYSIS

Following each test, breath-by-breath data were subjected to standard Fourier analysis. The analysis yielded the parameters of $\dot{V}O_2$ kinetics, i.e. phase delay and amplitude ratio, for a range of harmonics.

Assessments of intra-subject variability in phase delay and amplitude ratio were made using the analysis of variance (ANOVA) technique.

Limits of agreement (Altman 1991) were calculated in order to assess how well individual results obtained in the two tests agreed.

RESULTS

(i) Heart rate

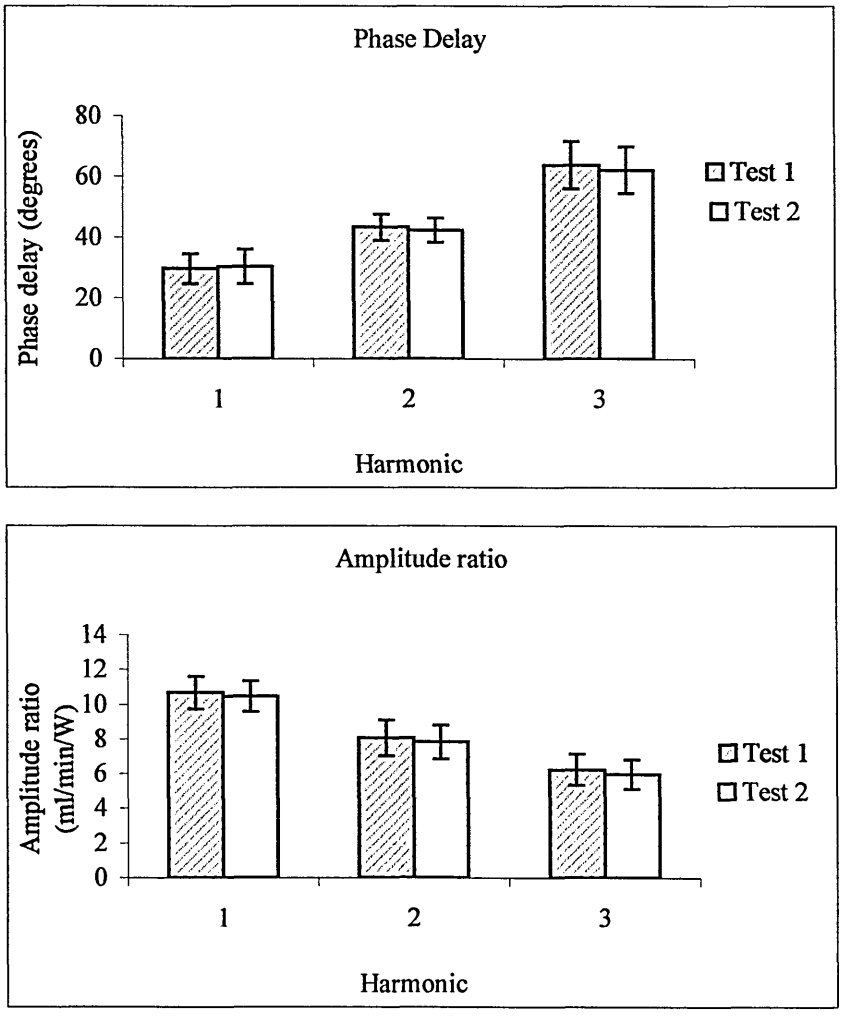
The mean heart rate recorded during the two tests was 102 ±11 beats·min^{-1}.

(ii) Oxygen uptake kinetics

Mean values for phase delay and amplitude ratio are shown in Figure 1.

ANOVA revealed no significant intra-subject variability between the two tests ($p<0.05$).

Figure 1: Mean values for the phase delay and amplitude ratio obtained during each test: mean values (±SD).



Wide 95% limits of agreement were observed at each harmonic for both phase delay and amplitude ratio (Table 1).

Table 1: 95% Limits of agreement (Test 2 minus Test 1): mean difference (±2SD).

Harmonic	1	2	3
Phase delay: mean difference (degrees)	+0.70 (±7.37)	-0.95 (±9.59)	-1.55 (±11.32)
95% limits of agreement (degrees)	-6.67 to +8.07	-10.54 to +8.64	-12.87 to +9.77
Amplitude ratio: mean difference ($ml \cdot min^{-1} \cdot W^{-1}$)	-0.21 (±1.42)	-0.23 (±0.88)	-0.27 (±0.98)
95% limits of agreement ($ml \cdot min^{-1} \cdot W^{-1}$)	-1.63 to +1.21	-1.99 to +1.53	-1.25 to +0.71

DISCUSSION

The purpose of this investigation was to assess the test / re-test reliability of a specific PRBS protocol.

Statistical analysis revealed no significant difference between the test and re-test data. Although this suggests that the PRBS protocol is reliable for group data, the wide limits of agreement indicate a large variability in individual $\dot{V}O_2$ kinetics.

The variability in $\dot{V}O_2$ kinetics for a given individual may be the result of technological error or biovariation (Katch 1992). By applying appropriate techniques it may be possible to overcome these influences and reduce variability to within acceptable limits.

CONCLUSION

From the evidence of this investigation it would appear that this particular PRBS protocol can be reliably used in the analysis of group $\dot{V}O_2$ kinetics. However, unless certain methods can be applied to reduce the relatively large individual variation, the interpretation of such data must be treated with caution.

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Appendix 4.1

Measurement of Oxygen Uptake Using Two Breath-by-Breath Gas Analysis Systems

Despite the limitations of biological calibrants (Chapter 2 Section 2.3.4.3), the measurement of $\dot{V}O_2$ on a cycle ergometer over a range of moderate work rates (eg. 50 to 150 watts) may provide a suitable baseline indicator of the performance of respiratory gas analysis systems. The aims of this experiment are:

- To quantify the oxygen uptake response during low intensity steady state exercise and,
- To compare the steady state oxygen uptake values at selected work intensities with published normative values

Equipment

The two respiratory gas analysis systems used in this investigation are a Marquette MGA1100, and a MedGraphics CPX-D. The Marquette system is based on a mass spectrometer medical gas analyser (Marquette Electronics Inc. Milwaukee, U.S.A.), a turbine flow meter (Ventilation Measurement Module model VMM-2A, Interface Associates, Laguna Niguel, CA, U.S.A.) for volume determination. The system is integrated, via an A/D converter, by an IBM compatible 80386 computer running First Breath Software version 2.0 (First Breath Inc. Ontario, Canada). The MedGraphics CPX-D (Medical Graphics Corporation Minnesota, U.S.A.) is based on two discrete gas analysers - Zirconia for O_2 and Infrared for CO_2, and a differential pressure transducer flow device (MedGraphics Disposable Pneumotach), interfaced with an IBM 286 computer.

Table 1. Major components of the Marquette MGA 1100 and MedGraphics CPX-D.

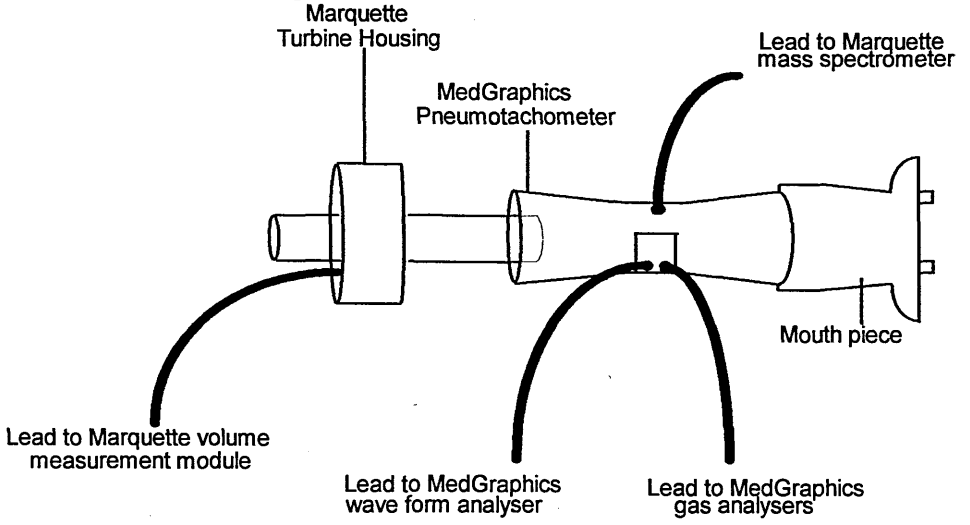
Component	Marquette MGA-1100	MedGraphics CPX-D
Gas Analyser(s)	Mass spectrometer 90° magnetic fixed collector N_2, O_2 and CO_2.	O_2 Zirconia, CO_2 Infrared
Flow Device	Turbine flow meter	Disposable Pneumotach

Table 1 provides a brief description of the major components of each system.

The two systems calculate and report respiratory and metabolic parameters on different breath-by-breath bases in terms of the calculations involved. The difference between the two systems is essentially the ability of the Marquette system to measure inspired and expired nitrogen. This additional parameter is purported to have three distinct advantages, 1) any discrepancies in the volume calibration may be detected as a result of a positive or negative nitrogen balance, 2) the ability to estimate alveolar gas

exchange and 3) the sensitivity to volume measurement error is small as a result of the calculation accounting for changes is in lung gas stores. For the Marquette system oxygen uptake is calculated using Chapter 2 equations 14-18 and nominal lung volume estimated as ½ FRC. Functional residual capacity was derived from tables of Taylor et al. (1989). The MedGraphics uses the Haldane transformation (Chapter 2 Equations 7-12).

Figure 1. Mouth piece arrangement for simultaneous measurement using the MedGraphics and Marquette gas analysis system



In order to remove the variability caused by physiological and environmental factors it is necessary to perform the analysis using both systems simultaneously. The simultaneous assessment required a minor modification of the mouthpiece assembly (Figure 1).

Subjects

Six healthy male subjects age 27.5 (± 9.5) years, height 1.82 (± 0.09) m and weight 72.5 (± 8.6) kg volunteered for the study. Values shown are mean (± standard deviation).

Protocol

Each subject performed 3 identical sub-maximal incremental cycle ergometer tests. The test was undertaken using an electrically braked cycle ergometer (ERG 550, Bosch GMBH, Berlin, Germany). Exercise was commenced at an initial work intensity of 50 W and increased by 25 W every 3 minutes until the subject had exercised for a total of 15 minutes, reaching the final intensity of 150 watts. A constant 1 Hz pedal frequency was maintained throughout the test. The First Breath Inc. (Marquette Electronics Inc. Milwaukee, USA) v 2.0 Work Rate Control Software was used to control the cycle ergometer.

Three identical tests were performed by each subject whilst monitoring was undertaken continuously using either the MedGraphics (MEDIND), Marquette (MARIND) or the

two gas analysis systems simultaneously (MEDSIM and MARSIM). The order of the type of monitoring used was randomised using a Latin Square design.

4) Statistical analysis.

All the physiological variables reported were calculated as the mean of the responses measured during the last minute at each work intensity:

a) Heart rate (HR)

b) Respiratory rate (RR)

c) Minute volume of ventilation or ventilation expired ($\dot{V}E$)

d) Oxygen uptake or $\dot{V}O_2$.

e) Respiratory Exchange Ratio (RER).

A two way analysis of variance (ANOVA) with repeated measures was used to determine the difference between the physiological responses measured by the two gas analysis systems at each of the five work intensities. Post hoc testing used the Tukey method. Significant differences were accepted if $P \leq 0.05$.

Where significant differences were observed between the oxygen uptake measured by the two systems during the simultaneous experiment, limits of agreement (mean difference ± 2 standard deviations) were calculated and linear regression analysis was used to compare the two systems.

Results.

Heart rate. There was no significant difference between the heart rates in the three exercise protocols. The final heart rates at 150 watts are shown in Table 4. They demonstrate the sub-maximal nature of the exercise.

Table 2 Average Heart Rate during final minute at 150 watts.

PROTOCOL	HEART RATE (Beats·min^{-1})	SD
MED(IND)	128	20.3
MAR(IND)	133	19.1
MED/MAR (SIM)	132	22.2

MED(IND) refers to the independent test on MedGraphics.
MAR(IND) refers to the independent test on Marquette.
MED/MAR (SIM) refers to the simultaneous test.

Oxygen Uptake and Ventilation Rate.

Averages of the last minute at each work intensity for oxygen uptake ($\dot{V}O_2$), ventilation ($\dot{V}E$) and respiratory rate (RR) for each of the analysis methods are presented in Tables 3, 4 and 5.

The results of a two way ANOVA with repeated measures showed that there was no significant difference between the two independent and the simultaneous measurement conditions for the parameters of $\dot{V}E$ and RR.

For $\dot{V}O_2$ there was a significant difference between trials ($P < 0.001$). A post hoc Tukey analysis indicated that the measurements made between the two systems, independently or simultaneously, were significantly different ($P < 0.05$). The MedGraphics system reported consistently lower values than that of the Marquette system.

Table 3 Oxygen Uptake Recorded Under 3 Measurement Conditions.

WORK RATE (Watts)	MEDIND ($ml \cdot min^{-1}$)	MEDSIM ($ml \cdot min^{-1}$)	MARIND ($ml \cdot min^{-1}$)	MARSIM ($ml \cdot min^{-1}$)
50	979 (91.4)	993 (101.9)	1084 (86.3)	1099 (70.5)
75	1133 (128.5)	1125 (112.1)	1276 (87.0)	1242 (87.7)
100	1381 (104.0)	1397 (77.8)	1500 (73.4)	1494 (47.4)
125	1615 (114.9)	1606 (99.8)	1774 (78.3)	1725 (44.8)
150	1905 (139.2)	1923 (130.1)	2089 (31.7)	2040 (51.6)

Values displayed = means (standard deviation).

Table 4 Ventilation Recorded Under 3 Measurement Conditions.

WORK RATE (W)	MEDIND ($l \cdot min^{-1}$)	MEDSIM ($l \cdot min^{-1}$)	MARIND ($l \cdot min^{-1}$)	MARSIM ($l \cdot min^{-1}$)
50	23.9 (2.14)	25.1 (2.76)	23.5 (2.87)	25.0 (2.99)
75	28.7 (3.69)	29.5 (3.34)	27.9 (2.84)	29.2 (3.40)
100	35.9 (5.56)	35.6 (4.30)	34.1 (4.12)	35.3 (3.84)
125	42.0 (7.31)	41.3 (6.59)	41.7 (7.42)	41.1 (6.32)
150	49.0 (9.49)	50.9 (7.70)	51.5 (8.75)	50.5 (7.23)

Values displayed = means (standard deviation).

Table 5 Respiratory Rate Recorded Under 3 Measurement Conditions.

WORK RATE (Watts)	MEDIND (breaths·min^{-1})	MEDSIM (breaths·min^{-1})	MARIND (breaths·min^{-1})	MARSIM (breaths·min^{-1})
50	19.8 (3.43)	19.8 (3.76)	19.0 (3.58)	19.7 (3.98)
75	21.7 (5.57)	22.33 (3.78)	20.8 (3.71)	22.2 (3.92)
100	23.0 (7.07)	22.8 (4.67)	21.8 (5.00)	22.8 (4.83)
125	23.8 (6.74)	23.8 (6.37)	23.3 (6.56)	24.0 (6.51)
150	26.5 (7.37)	26.0 (6.36)	26.3 (8.45)	26.0 (6.36)

Values displayed = means (standard deviation).

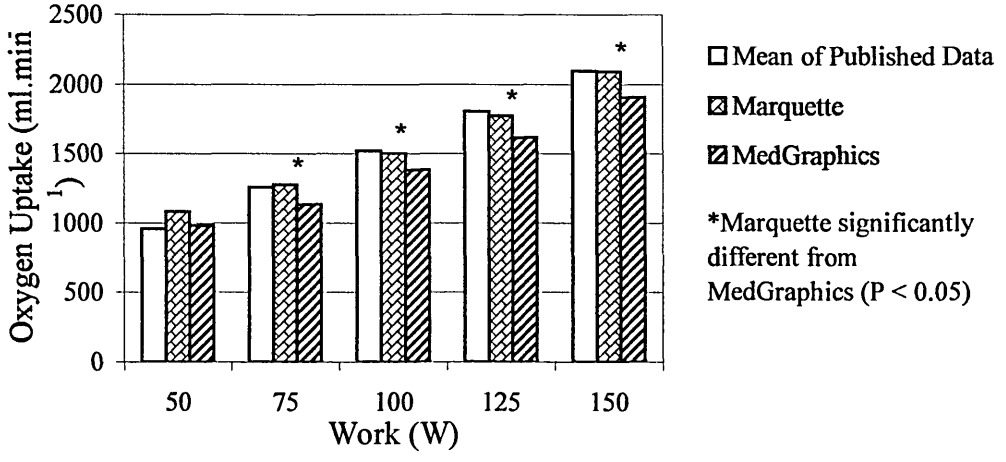
Mouth piece adaptation.

There was no significant difference between the independent and the simultaneous trials of each system, indicating that the modification made to the mouthpiece used to perform the simultaneous test had not compromised the data collection process of either system.

Comparison with normative data.

Since the normative values for $\dot{V}O_{2(ss)}$ varies between authors, the results from MedGraphics and Marquette were compared with the average data from the three sources (American College of Sports Medicine (ACSM), 1991; Åstrand and Rodahl, 1986 and Wasserman et al. 1987).

Figure 4 Oxygen Uptake Recorded by the Medgarphics and Marquette Gas Analysis Sytems Compared to Normative Data



The mean oxygen uptake values compared to normative values are shown in Figure 4.

It can be seen that apart from the lowest intensity work rate the Marquette system more closely reflects the normative values for $\dot{V}O_{2(ss)}$.

Respiratory Exchange Ratios.

The results from the simultaneous tests were analysed to determine which system gave the most predictable physiological results.

The final RERs at each work rate are presented in Table 6:

Table 6. RER value at the work intensity of 150 watts (mean for 6 subjects).

	RER				
WORK (W)	50	75	100	125	150
MARQUETTE	0.875 (0.031)	0.913 (0.055)	0.928 (0.035)	0.949 (0.055)	0.978 (0.034)
MEDGRAPHICS	0.891 (0.036)	0.933 (0.048)	0.955 (0.028)	0.979 (0.052)	1.015 (0.030)

Values displayed = means (standard deviation).

Due to the sub-maximal nature of the test (based upon the heart rate responses Table 4) the RER values would not normally be expected to exceed unity. The results demonstrate a potential overestimation of RER by the MedGraphics system at the highest work intensity of 150 watts..

Limits of Agreement.

The mean difference in the measured $\dot{V}O_2$ between the two systems is 111 ml·min^{-1} and the limits of agreement are +36 to -259 ml·min^{-1} which demonstrates the bias for the MedGraphics system to read lower than the Marquette.

Correction Factors to Compare MedGraphics and Marquette.

The relationship between the oxygen uptake measurements produced by the Marquette and MedGraphics system is described by the following linear regression equation:

$$\dot{V}O_{2\,(Marquette)} = 98 + 1.01 \times \dot{V}O_{2(MedGraphics)}$$

The adjusted R^2 of 0.999 a near perfect correlation indicates that throughout the range of measurement the difference between the systems is consistent. By forcing the regression line through the origin the relationship between the two systems is as follows:-

$$\dot{V}O_{2\,(Marquette)} = 1.08 \times \dot{V}O_{2(MedGraphics)}$$

The above equation, although not as accurate as using the full regression equation, indicates that the $\dot{V}O_{2(MedGraphics)}$ is on average 8% lower than the $\dot{V}O_{2\,(Marquette)}$.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	DATA RECORD FOR 20 CONSECUTIVE BREATHS - SUBJECT SHOWN IS 12 YEAR OLD FEMALE															
2			NLV equivalent to ½FRC =	885.2										Post hoc calculated ELV :	728	
3	$FETO_2$	INS_TIME	EXS_TIME	$FETN_2$	INS N_2	EXS N_2	INS O_2	EXS O_2	fb	I-E VO_2	I-E VN_2	delta $FETN_2$	delta $FETO_2$	deltaVL	VO_2 (Beaver et al.1981)	VO_2 $(n - (n-1))^2$
4	---	(s)	(s)	---	(ml)	(ml)	(ml)	(ml)	(breath.min^{-1})	(ml)	(ml)	---	---	(ml)	(ml.min^{-1})	---
5	0.1509	0.62	0.907	0.7825	314	348	83	72	39.29	11	-34					
6	0.1495	0.69	0.988	0.784	357	333	94	69	35.76	25	24	0.0015	-0.0014	29.21994	774	
7	0.1485	0.701	0.937	0.7835	343	316	91	65	36.63	26	27	-0.0005	-0.001	34.92515	789	222
8	0.1485	0.66	0.872	0.7847	345	383	91	78	39.16	13	-38	0.0012	0	-49.539	797	67
9	0.1522	0.645	0.857	0.7835	364	364	96	75	39.95	21	0	-0.0012	0.0037	1.114551	725	5286
10	0.1531	0.701	0.953	0.7827	342	305	90	63	36.28	27	37	-0.0008	0.0009	48.01606	689	1263
11	0.1509	0.711	0.862	0.785	338	335	89	69	38.14	20	3	0.0023	-0.0022	1.689515	814	15676
12	0.1497	0.741	0.942	0.7837	335	313	88	64	35.65	24	22	-0.0013	-0.0012	29.27909	730	7010
13	0.1497	0.66	0.983	0.784	312	351	83	72	36.52	11	-39	0.0003	0	-50.02336	675	3060
14	0.1504	0.685	0.973	0.7837	383	396	101	81	36.19	20	-13	-0.0003	0.0007	-16.30941	794	14143
15	0.1566	0.66	0.832	0.7817	415	379	109	80	40.21	29	36	-0.002	0.0062	47.91534	683	12336
16	0.1556	0.59	0.937	0.7808	331	363	87	77	39.29	10	-32	-0.0009	-0.001	-40.1448	667	258
17	0.1517	0.685	0.912	0.7825	335	308	88	64	37.57	24	27	0.0017	-0.0039	32.92383	821	23626
18	0.1546	0.69	1.28	0.781	379	372	100	78	30.46	22	7	-0.0015	0.0029	10.36052	557	69526
19	0.1492	0.983	0.711	0.7832	316	323	83	65	35.42	18	-7	0.0022	-0.0054	-10.98182	835	77156
20	0.1536	0.58	0.595	0.7825	347	387	91	81	51.06	10	-40	-0.0007	0.0044	-50.46723	743	8426
21	0.1585	0.625	1.169	0.7795	470	467	124	100	33.44	24	3	-0.003	0.0049	6.649297	648	8987
22	0.1321	0.66	3.72	0.7904	297	439	78	90	13.70	-12	-142	0.0109	-0.0264	-189.6913	442	42485
23	0.1419	0.751	0.862	0.7882	365	405	97	80	37.20	17	-40	-0.0022	0.0098	-48.71738	624	33190
24															sum of squares =	322717
25																
26	Variables shown in BLACK are measurements made by the gas analysis system.															
27																
28	The BLUE indicates calculated parameters.															
29																
30	MAGENTA is the calculated alveolar gas exchange based on the alorithm of Beaver et al. 1981.The initial values (not shown) rely on an estimate of nominal lung volume (nlv) displayed in															
31	BLACK at the top of the page and set equivalent to one half Functional Redsidual Capacity (½FRC).															
32																
33	The post-hoc calculation of effective lung volume (ELV) shown in RED is generated using Excel Solver (Microsoft Excel 97 SR-1, Microsoft Corporation, Redmond, WA, USA) to calculate the															
34	ELV which minimises the sum of squares value shown in GREEN.															
35																
36																

Formula for all calculated values are shown in Appendix 4.2a.

	I	J	K	L	M	N	O	P
2				Post hoc calculated ELV :		728		
3	fb	I-E VO_2	I-E VN_2	deltaFETN$_2$	deltaFETO$_2$	deltaVL	VO_2 (Beaver et al.1981)	VO_2 $(n - (n-1))^2$
4	(breath.min^{-1})	(ml)	(ml)	---	---	(ml)	(ml.min^{-1})	---
5	=60/(B5+C5)	=(G5-H5)	=(E5-F5)					
6	=60/(B6+C6)	=(G6-H6)	=(E6-F6)	=(D6-D5)	=(A6-A5)	=(K6-(N2*L6))/D6	=((J6)-(N6*A6)-(M6*N2))*I6	
7	=60/(B7+C7)	=(G7-H7)	=(E7-F7)	=(D7-D6)	=(A7-A6)	=(K7-(N2*L7))/D7	=((J7)-(N7*A7)-(M7*N2))*I7	=(O7-O6)^2
8	=60/(B8+C8)	=(G8-H8)	=(E8-F8)	=(D8-D7)	=(A8-A7)	=(K8-(N2*L8))/D8	=((J8)-(N8*A8)-(M8*N2))*I8	=(O8-O7)^2
9	=60/(B9+C9)	=(G9-H9)	=(E9-F9)	=(D9-D8)	=(A9-A8)	=(K9-(N2*L9))/D9	=((J9)-(N9*A9)-(M9*N2))*I9	=(O9-O8)^2
10	=60/(B10+C10)	=(G10-H10)	=(E10-F10)	=(D10-D9)	=(A10-A9)	=(K10-(N2*L10))/D10	=((J10)-(N10*A10)-(M10*N2))*I10	=(O10-O9)^2
11	=60/(B11+C11)	=(G11-H11)	=(E11-F11)	=(D11-D10)	=(A11-A10)	=(K11-(N2*L11))/D11	=((J11)-(N11*A11)-(M11*N2))*I11	=(O11-O10)^2
12	=60/(B12+C12)	=(G12-H12)	=(E12-F12)	=(D12-D11)	=(A12-A11)	=(K12-(N2*L12))/D12	=((J12)-(N12*A12)-(M12*N2))*I12	=(O12-O11)^2
13	=60/(B13+C13)	=(G13-H13)	=(E13-F13)	=(D13-D12)	=(A13-A12)	=(K13-(N2*L13))/D13	=((J13)-(N13*A13)-(M13*N2))*I13	=(O13-O12)^2
14	=60/(B14+C14)	=(G14-H14)	=(E14-F14)	=(D14-D13)	=(A14-A13)	=(K14-(N2*L14))/D14	=((J14)-(N14*A14)-(M14*N2))*I14	=(O14-O13)^2
15	=60/(B15+C15)	=(G15-H15)	=(E15-F15)	=(D15-D14)	=(A15-A14)	=(K15-(N2*L15))/D15	=((J15)-(N15*A15)-(M15*N2))*I15	=(O15-O14)^2
16	=60/(B16+C16)	=(G16-H16)	=(E16-F16)	=(D16-D15)	=(A16-A15)	=(K16-(N2*L16))/D16	=((J16)-(N16*A16)-(M16*N2))*I16	=(O16-O15)^2
17	=60/(B17+C17)	=(G17-H17)	=(E17-F17)	=(D17-D16)	=(A17-A16)	=(K17-(N2*L17))/D17	=((J17)-(N17*A17)-(M17*N2))*I17	=(O17-O16)^2
18	=60/(B18+C18)	=(G18-H18)	=(E18-F18)	=(D18-D17)	=(A18-A17)	=(K18-(N2*L18))/D18	=((J18)-(N18*A18)-(M18*N2))*I18	=(O18-O17)^2
19	=60/(B19+C19)	=(G19-H19)	=(E19-F19)	=(D19-D18)	=(A19-A18)	=(K19-(N2*L19))/D19	=((J19)-(N19*A19)-(M19*N2))*I19	=(O19-O18)^2
20	=60/(B20+C20)	=(G20-H20)	=(E20-F20)	=(D20-D19)	=(A20-A19)	=(K20-(N2*L20))/D20	=((J20)-(N20*A20)-(M20*N2))*I20	=(O20-O19)^2
21	=60/(B21+C21)	=(G21-H21)	=(E21-F21)	=(D21-D20)	=(A21-A20)	=(K21-(N2*L21))/D21	=((J21)-(N21*A21)-(M21*N2))*I21	=(O21-O20)^2
22	=60/(B22+C22)	=(G22-H22)	=(E22-F22)	=(D22-D21)	=(A22-A21)	=(K22-(N2*L22))/D22	=((J22)-(N22*A22)-(M22*N2))*I22	=(O22-O21)^2
23	=60/(B23+C23)	=(G23-H23)	=(E23-F23)	=(D23-D22)	=(A23-A22)	=(K23-(N2*L23))/D23	=((J23)-(N23*A23)-(M23*N2))*I23	=(O23-O22)^2
24							sum of squares =	=SUM(P7:P23)

Values for all calculations are shown in Appendix 4.2.

Appendix 4.3

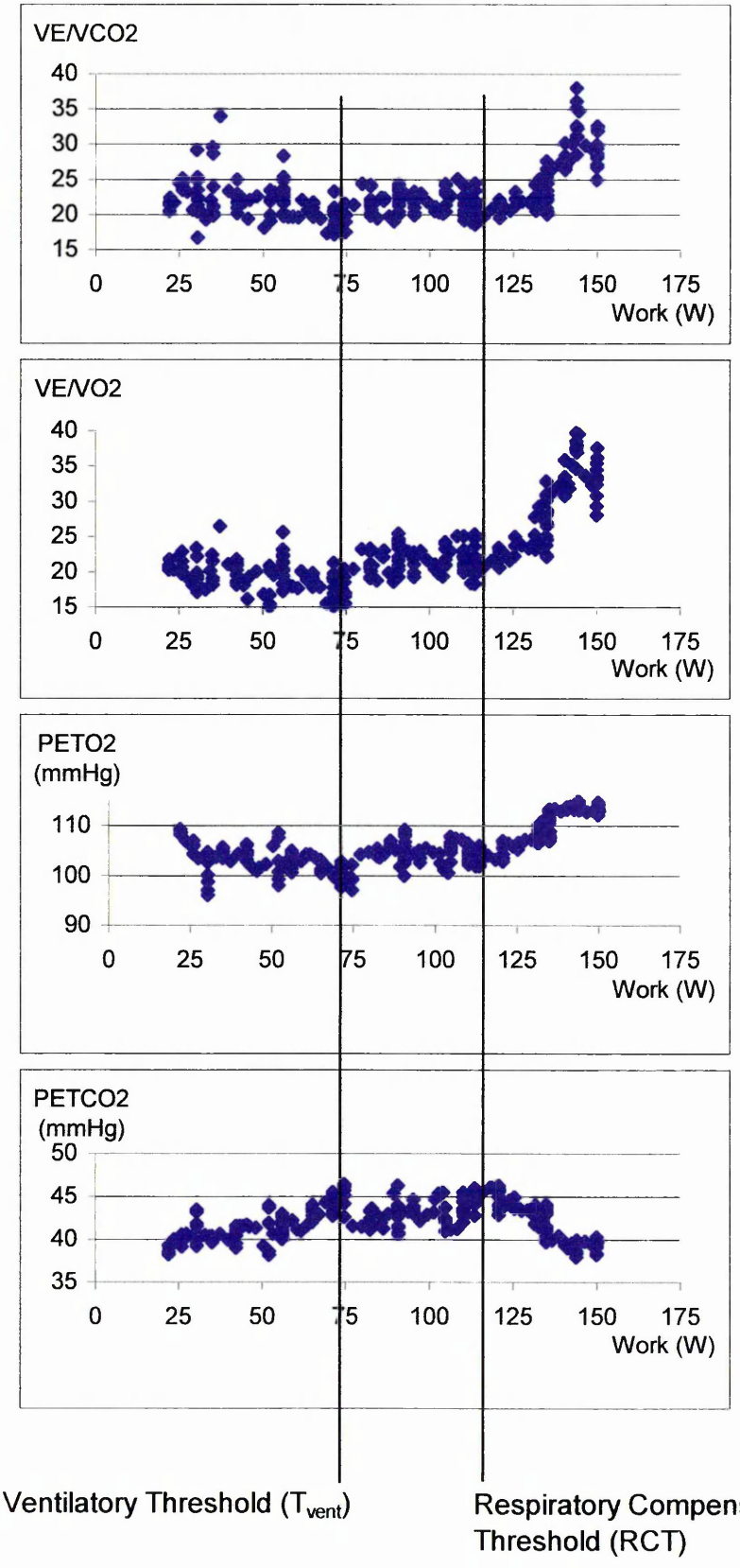
Correlation Program.

```
10  REM Auto- and Cross-correlation routine
20  REM ****************************************
30  CONST pi = 3.14159
100 DIM sig1(1000), sig2(1000), CCF(1000)
200 INPUT "Type 1 for signal data from file, 0 for test data"; ff
210 INPUT "Type number of data points per signal"; nmax
220 IF ff = 1 THEN
230     INPUT "Type filename...."; file$
240     OPEN file$ FOR INPUT AS #1
250     REM Here is the data input routine
260     REM nmax data points in arrays sig1 and sig2
265     REM sig1 and sig2 must BOTH have nmax points
270     REM ****************************************
300     FOR i = 1 TO nmax
310             INPUT #1, ii
315             PRINT "ii =", ii
320     NEXT i
330     FOR i = 1 TO nmax
340             INPUT #1, sig1(i)
345             PRINT "first signal = ", sig1(i)
350     NEXT i
360     FOR i = 1 TO nmax
365             INPUT #1, sig2(i)
370             PRINT "second signal = ", sig2(i)
375     NEXT i
380     CLOSE #1
390 ELSE
400     PRINT "Set up the test data points"
405     PRINT "t", "sig1", "sig2"
410     FOR i = 1 TO nmax
415             sig1(i) = 20
420     NEXT i
430     FOR i = 1 TO nmax
435             sig2(i) = 1000
450     NEXT i
452 REM         set up normalising functions
455     sum1 = 0
456     sum2 = 0
460     FOR i = 1 TO nmax
470             PRINT i, sig1(i), sig2(i)
471             sum1 = sum1 + sig1(i) * sig1(i)
472             sum2 = sum2 + sig2(i) * sig2(i)
480     NEXT i
490 END IF
491 REM normalise
492 sum1 = 0
493 sum2 = 0
494 FOR i = 1 TO nmax
495     sum1 = sum1 + sig1(i) * sig1(i)
496     sum2 = sum2 + sig2(i) * sig2(i)
497 NEXT i
498 sum1 = SQR(sum1)
499 sum2 = SQR(sum2)
500 REM ***************************
505 REM Now do cross correlation
510 REM ***************************
```

```
520 INPUT "Ready for cross-correlation?"; eh
535 PRINT "t", "CCF(t)"
540 FOR n = 1 TO nmax
550     sum = 0
555     FOR i = 1 TO nmax
556             it = i + n - 1
557             IF it > nmax THEN
558                     it = it - nmax
559             END IF
570             sum = sum + sig1(i) * sig2(it)
580     NEXT i
585     CCF(n) = sum / nmax
586     PRINT n, CCF(n)
590 NEXT n
700 REM ****************************
710 REM Now save to file for plotting
720 REM ****************************
800     INPUT "Type 1 to save to file, 0 to end"; ff
801     IF ff = 1 THEN
802             INPUT "Type filename...."; file$
803             OPEN file$ FOR OUTPUT AS #2
810             FOR i = 1 TO nmax
820                     PRINT #2, i
830             NEXT i
840             FOR i = 1 TO nmax
850                     PRINT #2, CCF(i)
860             NEXT i
891     END IF
940 END
```

Appendix 4.4

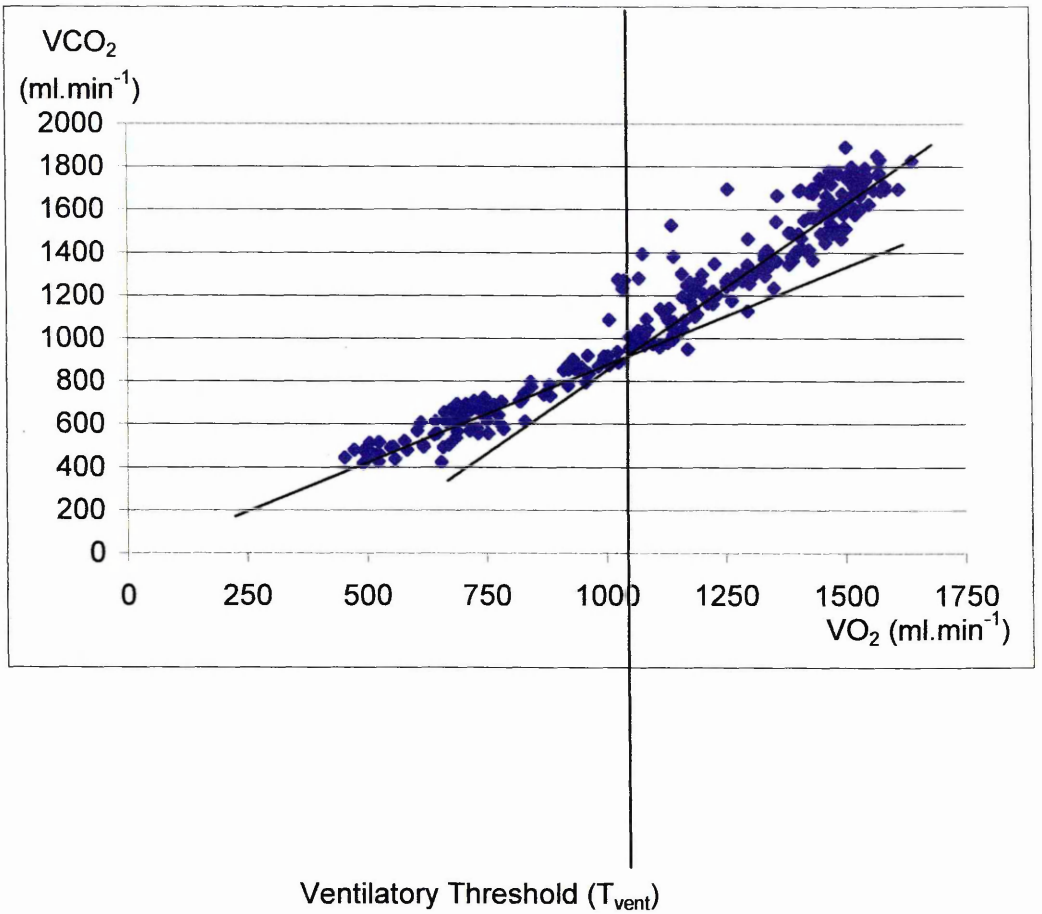
Subject : 10 year old male



See also Appendix 4.4a

Appendix 4.4a

V-slope method (Beaver et al., 1986)



Ventilatory Threshold (T_{vent})

See also Appendix 4.4

Appendix 5.1.

Pulmonary function, exercise performance, and growth in survivors of congenital diaphragmatic hernia

S S Marven, C M Smith, D Claxton, J Chapman, H A Davies, R A Primhak, C V E Powell

Abstract

A cohort of survivors of congenital diaphragmatic hernia (CDH), with matched controls, was studied to assess growth, respiratory function, and exercise performance. Nineteen of 24 survivors from an 11 year period (79%) were compared with 19 matched controls. Subjects had detailed auxology, performed spirometry and cycle ergometry, and completed questionnaires about respiratory symptoms and exercise. There were no significant differences between the groups for height, weight, sitting height, head circumference, or body mass index expressed as SD scores. The mean (95% confidence interval) percentage predicted forced vital capacity (FVC) was 84.7% (79.1 to 90.3) in index cases and 96.5% (91.4 to 101.6) in controls ($p < 0.01$). There was no significant difference in total lung capacity. Expiratory flow rates corrected for FVC were also similar between groups, suggesting normal airway function relative to lung size. Mean maximum oxygen consumption in ml/kg/min was 40.1 (36.8 to 43.4) and 42.2 (38.5 to 45.8) in index and control cases. These differences were not significant. Index cases achieved a similar minute ventilation to controls by more rapid and shallower breathing. Index cases had lower perception of their own fitness and lower enjoyment of exercise, although habitual activity levels were similar. Survivors of CDH repair have reduced functional lung volumes, but normal airway function compared with matched controls. They have no growth impairment nor significant impairment of exercise performance, although they have more negative perceptions of their own fitness. They should be encouraged and expected to participate fully in sport and exercise.

(*Arch Dis Child* 1998;78:137–142)

Keywords: congenital diaphragmatic hernia; exercise performance; growth; spirometry

Congenital diaphragmatic hernia (CDH) has significant mortality from pulmonary hypoplasia and from associated malformations.[1] Although there is evidence of persisting parenchymal abnormalities in the lungs of survivors,[2 7] their functional impact is unclear. Reports of lung function in childhood survivors have shown conflicting results.[2 4 8 10 11] Reduced lung volumes[2 8] and reduced expiratory flows[3] or both[9] have been reported, but others have reported no spirometric abnormalities.[4 10 11] Only one study[3] used matched controls. An increase in respiratory symptoms and intolerance of exercise have been reported,[3 8] but others have not found any continuing morbidity.[10 11] Subjective reporting of impaired exercise performance has been noted in some studies.[3 6 8 9] An uncontrolled study found normal values of oxygen uptake,[10] but a recent study has compared a group of CDH survivors with controls and shown reduced maximum oxygen consumption ($\dot{V}O_2max$) in index cases.[12] Growth in survivors of CDH has been studied in less detail, although there is a suggestion that such children may be underweight.[12 13] Our objective was therefore to examine growth, lung function, and exercise performance in a cohort of Sheffield survivors of CDH repair compared with matched controls.

Methods

SUBJECTS

Ethical permission for the study was granted by the South Sheffield research ethics committee.

Between 1978 and 1988, 48 patients were referred to the surgical neonatal unit in Sheffield with a diagnosis of CDH. Nineteen (79%) of 24 survivors were recruited. One patient had moved away and four were untraceable. Fifteen (79%) presented on the first day of life, 16 (84%) were left sided, and all survivors were operated on within 48 hours of presentation. Median duration of ventilation was four days (range one to 19). All were term deliveries. Mean birth weight of 3.27 kg (range 2.9 to 3.84). Four had recurrences requiring further surgery. Two had other surgery, one for a duodenal perforation and one for gastro-oesophageal reflux. One child had associated hydrocephalus and one had an atrial septal defect and pulmonary hypertension. Eighteen survivors were white and one was black Afro-Caribbean. Eleven were male. Mean age was 11.51 years (range 7.3 to 16.9) for survivors and 11.55 years (7.23 to 16.74) for controls. The control subjects were identified by a head teacher and were matched for age, gender, and race. A control was found for each survivor. Survivors and matched controls were studied on the same day under the same conditions.

GROWTH

Detailed auxology was performed by an experienced measurement technician. Standing and sitting heights were measured with a

Harpenden stadiometer, and body mass index (BMI) was calculated as weight/height^2. Pubertal staging was performed using Tanner's criteria.[14]

RESPIRATORY SYMPTOMS AND SPIROMETRY

All subjects were examined and asked to complete a standardised respiratory questionnaire with the help of their parent/guardian. The questionnaire contained the core questions from the ISAAC (international study of asthma and allergy in childhood) questionnaire.[15]

Spirometry (maximum expiratory flow–volume loops) and lung volume measurements (helium dilution) were made using a Morgan eight litre "TLC test" rolling seal dry spirometer. Each child received a short period of instruction from an experienced technician immediately before testing; tests were performed with the children standing and wearing nose clips. Forced expiratory variables (forced expiratory volume in one second (FEV_1), forced vital capacity (FVC)) were determined from the best of three reproducible manoeuvres[16] and expressed as percentages of predicted values for height based on data from Sheffield schoolchildren.[17] Helium dilution volumes were expressed as percentages of predicted values for height based on published norms.[18]

EXERCISE TESTING

Exercise was performed on an electrically braked cycle ergometer (Bosch ERG 550, Berlin, Germany). Electronic control of the ergometer ensures that the work intensity is independent of pedal speed. Respiratory gases were monitored on a breath by breath basis using a respiratory mass spectrometer (Marquette MGA1100, Marquette Electronics, Milwaukee, USA) and turbine flow meter (Interface Associates, Ventilation Measurement Module model VMM-2A). Cardiac frequency was recorded using a three lead electrocardiograph. The "First Breath v2.0" software (Marquette Electronics) integrated the signals from the mass spectrometer, flow meter, and electrocardiograph to provide on-line determination of metabolic and cardiorespiratory variables. The software also supplied the work rate control signal for the cycle ergometer.

Exercise protocols

Each subject performed a continuous incremental ramp protocol to maximum volitional exertion on the cycle ergometer in order to assess aerobic function.[19] Before starting the test, seat height and handlebars were individually adjusted and each subject was allowed a period of familiarisation with the respiratory apparatus and ergometer. To account for the variation in age, height, and weight of the test population, three different ramp protocols were used to ensure that the test would terminate after approximately 10 minutes of cycling.[20] Standing height was used as the criterion for determining the protocol to be undertaken.[21] We used a ramp protocol of 10 W/min for a height below 125 cm, 15 W/min for heights between 125 and 150 cm, and 20 W/min for taller children. Where the height of the index and matched control fell into different protocol categories, the protocol appropriate to the shorter child was used. The index and control subjects undertook the test on the same day, one after the other in a randomised order. All protocols began with unloaded cycling, after which the workload increased continuously at a rate equivalent to 10, 15, or 20 W/min. Subjects were asked to maintain a pedal frequency of 60 revolutions per minute throughout the exercise. Strong verbal encouragement was used to motivate the subject during the latter stages of the test, which terminated when the subject was unwilling or unable to continue.[22]

Analysis of exercise data

The final work rate was taken as the highest work rate achieved at test termination. Breath by breath respiratory data and heart rate were all expressed as 30 second averages.[23] The following variables were recorded at peak exercise: oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), minute ventilation (Ve), tidal volume (Vt), respiratory frequency (rf), fractional end tidal oxygen ($FETO_2$) and fractional end tidal carbon dioxide ($FETCO_2$). The time taken for inspiration (Ti) was calculated as a percentage of the total breath duration (Ttot). The tidal volume inspired (Vi) and the tidal volume expired (Ve) were expressed as a ratio of the time taken for inspiration and expiration respectively (Vi/Ti and Ve/Te). The $\dot{V}O_2$ plateau was defined as an increase in $\dot{V}O_2$ during the final stages of exercise of less than 2 SD below the mean increases in $\dot{V}O_2$ during previous submaximal work rates.[24] Subjects were judged not to have made an adequate effort if they failed to achieve any one of the following criteria: (1) RER > 1; (2) maximum heart rate within 2 SD of that predicted[21]; (3) plateau achieved in $\dot{V}O_2$.

ACTIVITY AND ATTITUDE QUESTIONNAIRES

Habitual activity questionnaire

The activity questionnaire was designed to identify, by interview, the amount of time spent by each child in low, moderate, and high intensity physical activities during the seven days preceding the visit to the laboratory.[25] A weekly activity score was calculated by summing the scores from each period of activity. The scores ranged from 1 to 7, where a score of 1 was assigned to a low intensity exercise period of less than 20 minutes and a score of 7 denoted a high intensity exercise for more than 20 minutes.

Perception and attitude questionnaires

A five point Likert scale[26] was used to establish the children's perception of personal fitness when compared to their peers. The scope of the response was limited to those walking, running, or cycling activities which had been identified in the habitual activity questionnaire. A similar five point scale was used to determine the child's general attitude towards exercise.

Table 1 Growth data in 18 children surviving CDH repair and matched controls

	CDH	Controls	Differences (index–control)
Height SD score	0.19 (−0.23 to +0.60)	−0.04 (−0.46 to +0.38)	+0.22 (−0.25 to +0.70)
Weight SD score	−0.30 (−0.99 to +0.40)	−0.18 (−0.71 to +0.33)	−0.11 (−0.73 to +0.51)
BMI SD score	−0.61 (−1.44 to +0.23)	−1.36*(−2.01 to −0.7)	+0.75 (−0.115 to +1.62)
OFC SD score	−0.74*(−1.4 to −0.07)	−0.22 (−0.72 to +0.28)	−0.55 (−1.34 to +0.24)
Sitting height SD score	−0.72 (−0.69 to +0.54)	−0.56 (−1.20 to +0.08)	−0.49 (−0.11 to +1.09)

Values shown are mean (95% confidence interval). BMI = body mass index; OFC = occipitofrontal head circumference.
*Significantly different from reference values ($p<0.05$).

Table 2 Reported respiratory morbidity in children surviving CDH repair and matched controls

Symptoms	Survivors	Controls
Ever wheezed	7 (37%)	5 (26%)
Wheezed in last 12 months	6 (32%)	3 (16%)
Severe wheeze limiting speech	1 (5%)	1 (5%)
Persistent cough more than weekly	1 (5%)	2 (11%)
Night cough more than weekly	2/18 (11%)	1/19 (6%)
Asthma ever	8 (42%)	3 (16%)
Current asthma	5 (26%)	4 (21%)
Exercise induced wheeze?	8 (42%)	4 (21%)
Sometimes	5	4
Most times	3	0
Ever had eczema	6 (31%)	4 (23%)
Ever had hayfever	3 (17%)	2 (11%)
Family history of atopy	10 (53%)	11 (58%)

Table 3 Percentage predicted lung function in children surviving CDH repair and matched controls

	Index	Control	Differences (index–control)
TLC	89.3 (83.3 to 95.4)	94.1 (90.0 to 98.2)	−4.8 (−12.0to+2.4)
FRC	94.7 (86.0 to 103.4)	97.6 (89.4 to 105.5)	−2.9 (−16.0to+10.2)
RV	101.0 (77.9 to 124.2)	77.0 (62.5 to 91.6)	+24.0 (−3.0 to+51.0)
RV/TLC		16.2 (13.0 to 19.3)	+5.3*(+0.20to+10.5)
(absolute ratio)	21.5 (17.5 to 26.3)		
FVC	84.7 (78.8 to 90.6)	96.5 (91.1 to 101.9)	−11.8†(−19.1to−4.5)
FEV_1	78.7 (72.5 to 84.8)	92.3 (86.7 to 97.9)	−13.6†(−22.1to−5.2)
FEF_{25-75}	69.0 (55.9 to 82.1)	83.7 (76.5 to 90.9)	−14.7*(−27.9to−1.6)
FEF_{25}	80.5 (69.2 to 91.9)	95.4 (87.1 to 103.7)	−14.9*(−29.5to−0.3)
FEF_{50}	77.4 (64.8 to 90.1)	90.9 (82.2 to 99.7)	−13.5 (−27.5to+0.5)
FEF_{75}	83.0 (68.7 to 97.3)	88.0 (78.0 to 97.9)	−5.0 (−19.7to+9.7)

Values shown are mean (95% confidence interval). TLC = total lung capacity; FRC = functional residual capacity; RV = residual volume; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; FEF = forced expiratory flow.
†$p<0.01$, *$p<0.05$ index cases compared with controls (paired t test).

Table 4 Forced expiratory flows (FEF) corrected for FVC in children surviving CDH repair and matched controls

	Index	Control	Differences (index–control)
FEF_{25}	1.67 (1.37 to 1.96)	1.69 (1.53 to 1.85)	−0.03 (−0.34 to +0.29)
FEF_{50}	1.24 (0.98 to 1.50)	1.24 (1.11 to 1.37)	0.00 (−0.21 to +0.21)
FEF_{75}	0.68 (0.52 to 0.84)	0.61 (0.53 to 0.69)	+0.07 (−0.06 to +0.20)
FEF_{25-75}	0.98 (0.75 to 1.21)	1.02 (0.91 to 1.12)	−0.04 (−0.23 to +0.15)

Units are FVC/sec. Values shown are mean (95% confidence interval).
There are no significant differences.

STATISTICAL ANALYSIS

Standard deviation scores for height, weight, sitting height, head circumference, and BMI were calculated from reference data.[27,28] Continuous data from survivors and controls were compared using paired t tests (two tailed). Questionnaire data were tabulated and described as the numbers were too few for statistical comparison. Simple regression analyses or two group t tests were used to determine relations between perinatal variables and lung function.

Perception and attitude (Likert) scores were compared categorically. Pearson product moment correlation was used to describe the relation between the activity scores and $\dot{V}o_2$max (ml/kg/min). The study was estimated to have a 90% power of detecting a difference of 10% in percentage predicted FVC at 5% significance, using 19 pairs.

Results

GROWTH

Six of the index cases had a chest deformity, consisting of pectus excavatum in four, of whom two had ventral asymmetry; Harrison's sulci and asymmetry in one case, and asymmetry alone in one case. An index case was receiving oxandrolone for pubertal delay, and he and his control were excluded from growth analysis. Data on head circumference were missing for one index case. Fortuitously, each index case was at the equivalent pubertal stage to his or her matched control. Growth data for the index and control groups are shown in table 1. There were no significant differences between the two groups. The head circumference standard deviation score for index cases was significantly below the normal value of 0 ($p < 0.05$), as was the BMI score for the control children ($p < 0.01$).

RESPIRATORY SYMPTOMS AND SPIROMETRY

Respiratory questionnaires were correctly completed and returned by 18 survivors and 17 controls. The results are shown in table 2. About twice as many of the index cases reported respiratory symptoms, but the numbers were too small to draw statistical conclusions. The spirometry results are shown in table 3. The FEV_1 and FVC were significantly lower in the index cases ($p < 0.01$), but no significant difference was seen in total lung capacity (TLC). The control group residual volume (RV) was significantly lower than predicted but did not differ significantly from that of the index cases. Neither the side of the hernia nor the duration of neonatal ventilation significantly predicted subsequent FEV_1 or FVC. Although early (FEF_{25}) and mid (FEF_{25-75}) forced expiratory flow rates were significantly lower in index cases, this difference disappeared when the flow rates were expressed in units of FVC/sec (table 4). This indicates that the observed differences in flows were due to lower functional lung volumes rather than airway obstruction

EXERCISE PERFORMANCE

Of the 19 pairs of children tested, three were excluded from analyses. One control subject was too small to cycle and two index subjects (one of whom had a heart defect) failed to make an adequate effort. There were no significant differences between the index children and their controls in any of the cardiopulmonary variables measured at exhaustion (table 5). All the children achieved RER > 1, while 94% of index children and 88% of controls achieved a maximum heart rate within 2 SD of that predicted. Only 38% of index children and 13% of controls achieved a plateau of oxygen uptake. Ventilation at maximum aerobic exercise was significantly different between the two groups. The signifi-

Table 5 Peak cardiorespiratory responses to the cycle ergometer exercise (n=16 pairs)

	Index	Control	Differences (index–control)
Final work rate (W)	136 (111–161)	147 (122–171)	−11 (−24 to +3)
$\dot{V}o_2$max (ml/kg/min)	40.1 (36.8–43.4)	42.2 (38.5–45.8)	−2.1 (−4.8 to +0.7)
$\dot{V}o_2$max (l/min)	1.52 (1.26–1.78)	1.66 (1.41–1.91)	−0.14 (−0.31 to +0.03)
RER	1.13 (1.10–1.17)	1.14 (1.11–1.18)	−0.01 (−0.03 to +0.01
HR (beats/min)	197 (191–202)	191 (184–198)	+6 (−2 to +13)

Values shown are mean (95% confidence interval). $\dot{V}o_2$max = maximum oxygen consumption; RER, respiratory exchange ratio; HR, heart rate.
No differences are significant.

Table 6 Average ventilatory variables at exhaustion for index and control groups (n=16 pairs)

	Index	Control	Differences (index–control)
Ve (l/min)	56 (47.5–64.5)	60 (52–68)	−4.3 (−9.0 to +0.4)
Vt (l)	1.04 (0.81–1.27)	1.23 (1.01–1.45)	−0.19* (−0.05 to −0.32)
rf (breaths/min)	56 (51–61)	50 (46–54)	5.9* (+0.8 to +11.1)
Ti/Tb (%)	50 (48–52)	52 (51–53)	−1.6 (−3.5 to +0.3)
Vi/Ti (l/s)	1.59 (1.33–1.85)	1.65 (1.42–1.88)	−0.07 (−0.2 to +0.06)
Ve/Te (l/s)	1.51 (1.29–1.73)	1.68 (1.45–1.91)	−0.17* (−0.33 to −0.01)

Values shown are mean (95% confidence interval). Ve = minute ventilation; Vt = tidal volume; rf = respiratory frequency; Ti/Tb = inspiratory percentage of breath cycle; Vi/Ti = mean inspiratory flow; Ve/Te = mean expiratory flow.
*$p<0.05$.

cantly reduced Vt in the CDH group was partially compensated by an increased frequency of breathing (table 6).

In spite of the differences in ventilation apparent between the CDH survivors and their controls there was no difference in the alveolar gas concentrations at maximum exercise. Mean (SD) $FETo_2$ values for index and control children were 0.16 (0.01) and 0.16 (0.01), while $FETco_2$ values were 0.054 (0.006) and 0.053 (0.005) respectively.

ACTIVITY AND ATTITUDE QUESTIONNAIRES

The activity scores were similar for the two groups with mean scores of 75 (43) and 79 (43) for index and control children respectively. There was a significant positive correlation ($r = 0.53$, $p < 0.01$) for the group as a whole between activity scores and $\dot{V}o_2$max. When completing the Likert scale on attitudes to exercise, none of the children reported that they disliked exercise. Sixty three per cent of the index children and 94% of the controls enjoyed exercising. With regard to self perception of fitness, the index children perceived their own fitness to be worse than the control group with 63% (95% confidence interval 39% to 87%) of the controls and 25% (5% to 45%) of the index subjects considering themselves fitter than their friends ($p < 0.05$).

Discussion

GROWTH

There are few data on growth in survivors of CDH. Zaccara *et al*[11] found normal height attainment but lower than expected weight in a subgroup of survivors. In survivors of extracorporeal membrane oxygenation, growth and nutrition was found to be progressively impaired over the first two years in CDH infants compared to those with other pathology,[12] but these children had more severe disease than our cohort, and included several with long term oxygen dependency and major feeding problems. Our subjects showed no significant growth impairment compared with their controls, and although both groups had one growth variable which differed from reference norms, in neither case was this of sufficient magnitude to suggest a biological significance.

RESPIRATORY SYMPTOMS AND SPIROMETRY

CDH is known to cause a varying degree of pulmonary hypoplasia,[29] and survivors have been shown to have persisting abnormalities in ventilation and perfusion in both lungs,[3 7] which might be expected to impair exercise performance. Nevertheless, pulmonary ventilation is not considered a limiting factor to aerobic power in healthy children.[30] In view of the reduced numbers of airway divisions in this condition, it is likely that reduction in lung volumes would persist in survivors, but there is less reason intuitively to expect airway obstruction. Although the spirometric abnormalities reported in previous work have been inconsistent, most studies were uncontrolled and have often involved a relatively small selected group from among a total population of CDH survivors.

Our study of 79% of survivors using matched controls has shown that the predominant spirometric impairment is in functional lung volumes. The apparent reductions in expiratory flows were merely a reflection of the reduction in FVC, and were eliminated by correcting for lung size. It is surprising that a concomitant reduction in TLC was not seen. Recent work from the Netherlands[31] has also observed reduced FEV_1 and normal TLC, with an increase in RV/TLC, although FVC results were not given. The index cases in our study also appeared to have a higher RV/TLC ratio, despite the lack of airway dysfunction, and it is possible that there is a permanent loss of lung elasticity leading to a degree of emphysema and smaller functional lung volumes.

Previous findings of emphysema on *x* ray would support this.[2 5] Similar findings of increased RV/TLC without airway obstruction have been observed in very low birthweight survivors.[32] They may represent gas trapping in emphysematous or overdistended alveoli, possibly due to loss of elasticity.

Mild increases in RV/TLC associated with normal airway function appear not to be associated with symptoms.[10] We observed more reported symptoms in index cases, but the numbers available for study preclude firm conclusions being drawn. The overall impact of the spirometric abnormalities in our cases did not appear to be severe.

EXERCISE PERFORMANCE, ACTIVITY, AND ATTITUDES

Our study had 90% power to detect a difference of 12.5% in mean $\dot{V}o_2$max/kg at 5% significance. We did not detect a significant difference in $\dot{V}o_2$max/kg, although there were differences in the mechanics of exercise performance, evidenced by shallower, more rapid breathing in index children to achieve similar maximum minute ventilation. These findings are compatible with the reduced functional lung volumes which we have demonstrated in the index children. Inspiration was

attenuated more noticeably, which may have increased the energy cost of breathing slightly. The different breathing patterns of the two groups did not affect alveolar gas concentrations. Vanamo *et al* showed that diffusing capacity was normal in CDH patients,[9] excluding the possibility of a significant disturbance in gas exchange at the level of the alveolar membrane.

The average aerobic power ($\dot{V}o_2max$) achieved by both groups is typical for normal children aged between 6 and 18 years. Armstrong and Davies[30] found a weight corrected $\dot{V}o_2max$ of between 38 and 42 ml/kg/min. The European Paediatric Work Physiology Group recommend a minimum aerobic power of 35 ml/kg/min for boys and 30 ml/kg/min for girls.[33] Only one child from the CDH group and no children in the control group fell below the recommended level of aerobic power so could be classified as unfit. The aerobic power demonstrated by the children surviving CDH repair and their controls means that on average these children should be capable of continuous running at speeds up to 10 km/hour.[34] The small difference of 2 ml/kg/min between the groups has very little functional significance in terms of exercise or sporting performance.

A vexed question in the study of exercise in children is whether a maximum level has been reached. This study identifies specific criteria for maximum oxygen uptake. Strong verbal encouragement was given to each child but because of the way in which the cycle ergometer increases the resistance once the cadence drops, it is difficult for a child to have enough leg strength to resume the exercise if the pedal frequency becomes too low. In order to quantify maximum effort, either heart rate or respiratory exchange ratio can be used. Another criterion is a plateau in oxygen uptake, even though the work rate is still increasing, although some investigators[21] argue strongly against the use of the plateau method of defining maximum effort in children. As expected[31] only a minority of children in this study showed a plateau, but more CDH survivors were in this category than control children. Similarly the group of index children reached higher heart rate at exhaustion (197 (9.8) beats/min) than the control group (191 (13.1)), which suggests that it is likely that maximum effort was obtained. Average respiratory exchange ratios of 1.13 and 1.14 for CDH and control groups, respectively, are again indicative of maximum effort and support the view that strong verbal encouragement is required if maximum values are to be achieved.[10]

Of the two most recent studies, Vanamo *et al* described a subjective intolerance in 13% of survivors,[9] many of whom were adults when investigated. Zaccara *et al* studied 15 children of similar mean age to the Sheffield children (11 years 3 months) by exercising them to exhaustion on a treadmill,[11] using a walking/running protocol. They found that their CDH patients had significantly lower aerobic capacities than their control group. They explained the low $\dot{V}o_2max$ of 31 ml/kg/min by the low exercise involvement of some of the CDH group. This was due in part to the extensive medical screening required by Italian law before children are permitted to participate in sport. Other explanations may lie in the fact that the Italian children were underweight for their age and also that they may not have reached maximum effort. On average, maximum heart rates of only 187 beats/min were recorded by these investigators. A similar problem occurred in the study of Freyschuss *et al*, where maximum mean heart rates of 186 beats/min and mean RERs of 0.98 were reported.[10] It is unlikely that the subjects studied by these workers achieved maximum effort.

One follow up investigation of CDH survivors has linked physical fitness with activity levels,[13] showing significant differences in $\dot{V}o_2max$ (l/min) between CDH patients who were sports participants and those who were sedentary. For the Sheffield children, no difference could be shown between the children surviving CDH and their controls. This may be partially explained by the fact that the children and their controls came from the same school class and experienced the same physical education curriculum. Other investigators of healthy children have shown a tenuous link between physical activity and physical fitness[35 36] and have concluded that inherited factors are more important in determining $\dot{V}o_2max$ than environmental factors. It was interesting therefore that both the control group and the CDH survivors showed significant positive correlations between $\dot{V}o_2max$ (expressed as ml/kg/min) and activity scores ($r = 0.52$ and $r = 0.64$ for controls and CDH group, respectively).

We considered how CDH survivors viewed their personal fitness. When asked to rate themselves in terms of fitness and also in terms of their attitude to exercise, the children surviving CDH tended to consider themselves as less fit than their peers and they also had a more negative attitude towards exercise in general. This is an interesting finding in view of the previous reports of subjective exercise intolerance, some of which may have been related to attitude and perception rather than to objective impairment. If the children surviving CDH repair are to achieve their full potential, it is essential that parents, teachers, and doctors encourage fuller participation in an active lifestyle, not only because it improves the general health of children[37] but also because the exercise habits of childhood are likely to be maintained into adult life.[38]

The neonatal management of congenital diaphragmatic hernia is changing. Preoperative stabilisation identifies those infants most likely to do well. In addition, advances such as high frequency oscillation and extracorporeal membrane oxygenation offer survival to those with more severe lung hypoplasia.[12 39] Our cohort survived a period of less sophisticated neonatal care, and is likely to represent a milder form of CDH than those who might be reported in the future. However, we can conclude that children with CDH who do not require such intensive intervention can be expected to have reduced

functional lung volumes but normal growth and relatively minor functional effects. In addition, the degree of exercise impairment observed in the CDH survivors has little if any functional significance. In the absence of associated abnormalities the children should be expected to achieve normal exercise function. We consider it unlikely, therefore, that children surviving CDH are adversely affected to any great extent by their condition, and we suggest that they should be encouraged to participate more fully in all forms of exercise and sport.

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Appendix 5.2

Child Information Sheet

Who will perform the study ?

Craig Smith is a Doctor working at Sheffield Children's Hospital. David Claxton is a scientist working at the Sheffield Hallam University. Craig and David will explain how to do the tests and show you what to do. They have performed these tests on many children before.

Why do the study?

To compare the results of your tests with those of your friend who had an operation when (s)he was a baby.

Where will the study take place?

In the Respiratory Function Laboratory, the Growth and Measurement Laboratory and the Radiology and Imaging Department in the Sheffield Children's Hospital. Also in the Exercise Physiology Laboratory of Sheffield Hallam University.

What will be involved if we agree to take part in the study?

Every effort will be made to make this an enjoyable a day as possible. We would like you to come with a friend from school for the following growth and fitness tests :-

Respiratory Function Laboratory

You will be asked to breathe into a machine to measure how big your lungs are.

Growth and Measurement Laboratory

You will be weighed and measured then examined by a doctor and asked some questions.

Radiology Imaging Department

An X ray of your left hand will be done. This will tell us how well grown your bones are.

Exercise Physiology Laboratory

You will be asked to pedal on an exercise cycle for 17 minutes, then after a short rest for a further 10 minutes whilst breathing into a tube.

Will there be any effects on me ?

These tests and measurements are safe. They do not hurt and they are not difficult and we expect that you will enjoy the exercises.

Will you tell any of my friends of family about the results ?

All information will be treated in strictest confidence.

Will you tell anyone else about my participation in the study ?

No names will be mentioned and care will be taken so that individuals cannot be identified from details in reports or from the results of the study.

What if I decide to withdraw from the study ?

Most children enjoy the time in our laboratories. If you decide that you do not wish to continue in the study you can stop and go home at any time.

Child Consent Form

The child should complete the whole of this sheet himself / herself.

Have you read the information sheet ? YES/NO

Have you had the chance to ask questions about this study ? YES/NO

Do you understand the answers to all of your questions ? YES/NO

Have you received enough information about the study ? YES/NO

Have you spoken to Dr Craig Sharp or Mr Sean Marven ? YES/NO

Do you understand that you are free to stop and go home :

at any time

without having to give a reason

without affecting your future medical care YES/NO

Do you agree to take part in this study ? YES/NO

NAME (IN BLOCK CAPITALS).....

Signed..... Date.....

Parent / Guardian Consent Form

The parent(s) should complete the whole of this sheet himself / herself (thenselves).

Have you read the information sheet ? YES/NO

Have you had the chance to ask questions about this study ? YES/NO

Do you understand the answers to all of your questions ? YES/NO

Have you received enough information about the study ? YES/NO

Have you spoken to Dr Craig Sharp or Mr Sean Marven ? YES/NO

Do you understand that you are free to withdraw your child :

at any time

without having to give a reason

without affecting your future medical care YES/NO

Do you agree to your child taking part in this study ? YES/NO

NAME (IN BLOCK CAPITALS).....

Signed..... Date.....

Appendix 5.3

Physical Activity Readiness Questionnaire (modified)

Exercise poses a minimal risk to healthy individuals. The following questionnaire has been designed to identify the small number of adults for whom physical activity may be inappropriate.

Complete this questionnaire before undertaking any physical activity in the laboratory.

Has your doctor ever said you have heart trouble ? YES/NO

Do you frequently suffer from pains in the chest ? YES/NO

Do you often feel faint or have spells of severe dizziness ? YES/NO

Has a doctor ever told you that you have a bone or joint problem such as arthritis that has been aggravated by exercise or might be made worse by exercise ? YES/NO

Are you suffering from a cold or other viral infection ? YES/NO

Do you have a musculo-skeletal injury which may be aggravated by exercise ? YES/NO

Is there a good physical reason not mentioned here why you should not exercise even if you wanted to ? YES/NO

If you answer YES to any of these questions please see a member of staff.