Energy expenditure and physical activity patterns in children: Applicability of simultaneous methods.

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KEYWORDS

Children, Energy metabolism, Indirect calorimetry, Resting Metabolic Rate, Self-selected speed, Measurement, Treadmill walking, Adaptation, Protocol, Oxygen cost of exercise, Exercise prescription, Substrate oxidation, Respiratory quotient, Reproducibility, Variability, Accelerometry, Heart rate monitoring, Activity energy expenditure, Total energy expenditure, Physical activity pattern, Metabolic equivalent

ABSTRACT

Consistently, reports in the literature have identified that a sedentary lifestyle contributes to the progression of a range of chronic degenerative diseases. The measurement of energy expenditure and physical activity pattern in children is a challenge for all professionals interested in paediatric health and from a broader perspective, the public health fraternity charged with considering longer term health consequences of physical inactivity. The primary objective of this thesis was to identify a suitable indirect and objective measurement technique for the assessment of energy expenditure and physical activity pattern in children. The ideal characteristics of such a technique are that it should be reproducible and have been validated against a criterion reference method. To achieve this goal, a series of methodological studies were undertaken (Chapters II and III). This work was essential to increase accuracy during the individualised laboratory calibration process and further minimise prediction errors when analysing data from 7 days of monitoring under free-living conditions in the second part of the study (Chapters IV and V).

In the first study to verify the combined effect of body position, apparatus and distraction on children's resting metabolic rate (RMR), experiments were carried out on 14 children aged 8-12 (mean age = 10.1 years ± 1.4). Each participant underwent 2 test sessions, one week apart under three different situations: a) using mouthpiece and nose-clip (MN) or facemask (FM); b) sitting (SEAT) or lying (LY) and c) TV viewing (TV) or no TV viewing. In the first session, following 20 min rest and watching TV, the following protocol was used: LY: 20 min – stabilisation; 10 min using MN and 10 min using FM. Body position was then changed to seated: 20 min stabilisation; 10 min using FM; 10 min using MN. In the second session, FM and MN order was changed and participants did not watch TV. Data were analysed according to the eight combinations among the three studied parameters. Repeated measures ANOVA indicated statistically significant differences for $\dot{V}O_2$ (p=0.01) and RMR (p=0.02), with TVMNSEAT showing higher values than TVFMLY. Bland-Altman analysis showed a bias for $\dot{V}O_2$, $\dot{V}CO_2$, RQ and RMR between TVFMLY and TVMNSEAT of -17.8±14.5 $ml.min^{-1}$, -8.8±14.5 $ml. min^{-1}$, 0.03±0.05

and -115.2±101.9 $kcal.d^{-1}$, respectively. There were no differences in RMR measurements due to body position and apparatus when each variable was isolated. Analyses of distraction in three of four combinations indicated no difference between TV and no TV. In summary, different parameter combinations can result in increased bias and variability and thereby reported differences among children's RMR measurement.

The second study dealt with treadmill adaptation and determination of self-selected (SS) walking speed. Assessment of individual and group differences in metabolic energy expenditure using oxygen uptake requires that individuals are comfortable with, and can accommodate to, the equipment being utilised. In this study, a detailed proposal for an adaptation protocol based on the SS was developed. Experiments were carried out on 27 children aged 8-12 (mean age = 10.3±1.2 yr). Results from three treadmill tests following the adaptation protocol showed similar results for step length with no significant differences among tests and lower and no statistically significant variability within- and between-days. Additionally, no statistically significant differences between SS determined over-ground and on a treadmill were verified. These results suggest that SS speed determined over-ground is reproducible on a treadmill and the 10 min familiarisation protocol based on this speed provided sufficient exposure to achieve accommodation to the treadmill.

The purpose of the third study was to verify within- and between-day repeatability and variability in children's oxygen uptake ($\dot{V}O_2$), gross economy (GE) [$\dot{V}O_2$ divided by speed] and heart rate (HR) during treadmill walking based on SS. 14 children (mean age = 10.2±1.4 yr) undertook 3 testing sessions over 2 days in which four walking speeds, including SS, were tested. Within- and between–day repeatability was assessed using the Bland and Altman method and coefficients of variability (CV) were determined for each child across exercise bouts and averaged to obtain a mean group CV value for $\dot{V}O_2$, GE and HR per speed. Repeated measures ANOVA showed no statistically significant differences in within- or between-day CV for $\dot{V}O_2$, GE or HR at any speed. Repeatability within and between-day for $\dot{V}O_2$, GE and HR for all speeds was verified. These results suggest that submaximal $\dot{V}O_2$ during treadmill walking is stable and reproducible at a range of speeds based on children's SS.

In the fourth study, the objective was to establish the effect of walking speed on substrate oxidation during a treadmill protocol based on SS. Experiments were carried out on 12 girls aged 8-12 (mean age = 9.9±1.4 yr). Each participant underwent 2 test sessions, one week apart. Workloads on the treadmill included 2 speeds slower than SS (1.6 [V1] and 0.8 $km.h^{-1}$ [V2] slower than SS), SS (V3), and a speed 0.8 $km.h^{-1}$ faster than SS (V4). Indirect calorimetry from respired gas measurements enabled total fat (FO) and carbohydrate (CHO) oxidation rates to be calculated according to the non-protein respiratory quotient (Peronnet and Massicote, 1991) and percentage of CHO and FO calculations using equations from McGilvery and Goldstein (1983). Repeated measures ANOVA followed by a *Tukey Post Hoc* test ($p<0.05$) was used to verify differences in CHO and FO rates among speeds. Paired T-test was used to verify differences in CHO and FO rates between tests per velocity. The reliability between–day was assessed using intraclass correlation coefficient (ICC). Results showed significant differences for CHO among all speeds, as well as significant differences for FO between V1 and V2 against V3 and V4 in both tests. Analyses between trials per velocity showed no significant substrate use differences as well as acceptable reliability. At the self-selected speed (V3) there was an accentuation in FO reduction as well as an increase in CHO oxidation.

The purpose of the fifth study was to determine whether there were differences in substrate oxidation between girls (G) and women (W) during a treadmill protocol based on SS. Experiments were carried out on 12 G aged 8-12 (mean age = 9.9±1.4 yr) and 12 W aged 25-38 (mean age = 32.3±3.8 yr). The treadmill protocol included 6 min workloads followed by 5 min rest periods. Workloads included 2 speeds slower than SS (1.6 (V1) and 0.8 $km\cdot h^{-1}$ (V2) slower than SS), SS (V3), and a speed 0.8 $km\cdot h^{-1}$ faster than SS (V4). Total fat and carbohydrate (CHO) oxidation rates were calculated from indirect calorimetry according to the non-protein respiratory quotient. Repeated measures ANOVA followed by a *Tukey Post Hoc* test was used to verify intra-test differences in CHO and fat oxidation rates among speeds. Inter-group differences were analysed using paired T-test. Fat utilisation in W achieved a plateau at a relative velocity 0.8 km·h^{-1} slower than SS, but for G, fat utilisation increased until SS, and then stabilised upon reaching the higher velocity. CHO oxidation curves rose abruptly above V2 for W, while for G the acute increase occurred after SS (V3). Collectively, these results indicate that as walking intensity increases G are able to meet the energy demands of the work by increasing fat oxidation together with the increased CHO oxidation up to SS. In contrast for W, increasing CHO oxidation is associated with an early decrease in fat utilisation at a velocity slower than the self-selected speed.

The sixth study dealt with validation of indirect techniques for the measurement of energy expenditure in free-living conditions against the DLW technique. Experiments were carried out on 19 children aged 8-12 (mean age = 10.3±1.0 yr). To indirectly predict energy expenditure 12 different procedures were used. Only one procedure, combining activity and heart rate ($AH_{branched}$), was based on a group equation, the others were based on individualised regression. Three of the individually-based techniques were able to accurately predict energy expenditure in free-living conditions. These tecniques were $HRPAnet_{RMR}$ using HR_{net} [HR exercise minus sleep HR (SHR)] against PA_{net} (measured PA exercise minus measured RMR) and upper and lower body equations corrected by RMR; $HRPAnet_{4act}$ using the same procedure but corrected by the mean resting $\dot{V}O_2$ for 4 resting activities [(4act) = supine watching TV, sitting watching TV, sitting playing computer games and standing], and $HRPALBnet_{4act}$ using only lower body activities and corrected by 4act. $HRPAnet_{RMR}$ was only slightly more accurate than $HRPAnet_{4act}$ and $HRPALBnet_{4act}$, but this technique is only adjusted by RMR whereas the other two are heavily dependent on more complex laboratory calibration. Bland and Altman (1986) analyses showed no significant differences between $AH_{branched}$ predicted and measured TEE using the DLW technique. A SEE of 79 kcal.d^{-1} and a mean difference of 72 kcal.d^{-1}, with a 95% CI ranging from -238 to 93.9 kcal.d^{-1} was found. In addition, no significant differences between predicted $HRPAnet_{RMR}$ and measured TEE using DLW were found, showing an SEE of 99 kcal.d^{-1} and a mean difference of -67 kcal.d^{-1}, and a 95% CI ranging from -276.6 to 141.9 kcal.d^{-1}. $AH_{branched}$ and $HRPAnet_{RMR}$ were both valid and similarly suitable for the prediction of energy expenditure in children under free-living conditions. Significant associations between DLW_{AEE} and the after-school time window indicated that this time window as an important discretionary period representative of children physical activity. However, the duration of the after-school time windows should be more carefully considered. Accelerometer data showed a better association between the largest after-school time window (3.5 hr) and measured TEE.

The final study, completed with 19 children aged 8-12 (10.3±1.0 yr) highlighted, under laboratory conditions across a range of walking and running speeds, the inadequacy of the use of the standard MET in children. This traditional approach overestimates energy expenditure with an increased difference linearly related to speed increments. Minute-by-minute analyses of 7 days of free-living monitoring showed an average overestimation of 64 minutes per day for moderate-to-vigorous-physical-activity (MVPA) using the standard MET compared with the individually measured MET. For all intensities, these differences were statistically significant ($p<0.001$). The second part of this study showed a variability of 20% in the average time spent at MVPA when comparing HR $\geq$ 140 bpm and HR > $_{50\%}P\dot{V}_{O2}$ ($P\dot{V}_{O2}$ = the highest $\dot{V}_{O2}$ observed during an exercise test to exhaustion). Results of the current study compared to observations in the literature showed that HR $\geq$ 140 bpm consistently estimates lower MVPA time than HR > $_{50\%}P\dot{V}_{O2}$. When these two PA indices were compared with individual and standard MET measured minute-by-minute, statistically significant differences were verified among all of them at MPA, but no differences were verified at VPA, except between individual and standard METs. However, whether each one of the PA indices used are under- or overestimating time at MVPA is still debatable due to the lack of a gold standard. Finally, each index used in this study classified different numbers of participants as achieving the PA target of 60 $min.d^{-1}$. The wide variability between indices when attempting to classify children who are achieving the recommended target is cause for great concern because habitually these indices are utilised as screening tools in paediatric and public health settings and used to guide behavioural interventions.

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Amorim PRS, Byrne NM, Hills AP. 'Comparison of substrate oxidation and body-size related performance in girls and women during treadmill walking.' (Under review)

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LIST OF ABBREVIATIONS

AB	Actiband
AEE	Activity energy expenditure
AH	Actiheart
BMI	Body mass index
CHO	Carbohydrate
CPM	Counts per minute
CV	Coefficient of variation
DLW	Doubly-labelled water
FFM	Fat-free mass
FM	Facemask
GE	Gross economy
HR	Heart rate
HR_{net}	Heart rate exercise minus sleep heart rate
IC	Indirect calorimetry
ICC	Intraclass correlation coefficient
LB	Lower body
MDPA	Mean daily physical activity
MET	Metabolic equivalent
MN	Mouthpiece and nose-clip
MPA	Moderate physical activity
MVPA	Moderate-to-vigorous physical activity
PA	Physical activity
PA_{net}	Measured physical activity exercise minus measured rest
PAL	Physical activity level
$P\dot{V}O_2$	(Volume) Peak oxygen uptake
REE	Resting energy expenditure
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
RQ	Respiratory quotient
RMR	Resting metabolic rate
SEE	Sleep energy expenditure

SHR	Sleep heart rate
SS	Self-selected speed
TEE	Total energy expenditure
UB	Upper body
$\dot{V}CO_2$	(Volume) carbon dioxide production
$\dot{V}O_2$	(Volume) oxygen uptake
VPA	Vigorous physical activity

STATEMENT OF ORIGINAL AUTHORSHIP

The work contained in this thesis has not been previously submitted for a degree or diploma at any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signed:
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Chapter I - INTRODUCTION

Thesis overview

This thesis is comprised of four sequential original studies, organised by chapters, with the main focus on the measurement of energy expenditure. This brief introduction presents an overview of some of the major concerns regarding children's physical activity from a public health perspective.

In Chapter II, the influence of different combinations of three methodological parameters – breathing apparatus, body position and distraction on resting metabolic rate (RMR) in children are discussed. Understanding the combined effect of these variables on the variability in RMR measurements has implications for the development of more accurate protocols to measure RMR in children.

Chapter III contains 4 sequential articles describing characteristics of some of the determinants of walking energy expenditure. Each article has been submitted for publication and all arecurrently under review. The purpose of the first study was twofold, firstly the reproducibility of the self-selected speed was determined using an over-ground protocol on the treadmill. Further, we proposed a 10 min familiarisation protocol able to verify the achievement of the self-selected speed and to assist in the improvement of the reliability of the oxygen uptake measures between tests. In the second study we investigated the within- and between-day repeatability and variability of oxygen uptake, gross economy and heart rate during treadmill walking using the self-selected speed and three derived velocities. The purpose of the third study was to describe the relationship between substrate oxidation and exercise intensity expressed as velocity based on the self-selected walking speed in girls. In the final study of this chapter the objective was to determine whether there are differences in oxygen uptake and substrate oxidation between girls and adult women during exercise with intensities based on self-selected walking speed.

The main purpose of Chapter IV was to verify the effectiveness of the different techniques utilised such as accelerometer, heart rate monitor and the simultaneous use of both, in a variety of calibration equations from low to high intensity activities, considering upper-body and lower-body activity predominance. Further, we controlled for the elevation in heart rate due to non-exercise reasons using a branched equation. Filters were inserted for data cleaning and the approach was validated against the doubly labelled water (DLW) technique as an alternative to energy expenditure prediction in free-living monitoring in children.

Chapter V involved the application of an individual regression equation validated against the DLW technique (as detailed in Chapter IV). This was undertaken to verify minute-by-minute energy expenditure expressed as multiples of measured resting energy value and analysed across 7 days of monitoring and quantified by intensity categories and compared with the standard value of 3.5 $ml.kg^{-1}.min^{-1}$ or 1 metabolic equivalent in children. In the second part of this chapter, three of the most common heart rate 'cut-offs' were applied together with the measured metabolic equivalent (MET) throughout 7 days of monitoring. Minute-by-minute data analyses were used to verify the agreement among them describing physical activity pattern as well as classifying children using the actual target of 1 hour of moderate-to-vigorous physical activity daily.

Finally, in the general discussion conclusions about key research questions, implications for theory and practice as well as future opportunities are highlighted.

Background

In modern industrialised societies, food and drink are more available and affordable than ever before, fewer people have jobs requiring hard physical labour, car ownership has increased rapidly and homes commonly have labour-saving devices. However, human energy metabolism has evolved under very different conditions with a sparse and often erratic food supply and significant physical demands for survival, which has selected individuals with a 'thrifty' genotype. This genotype is ill-suited to the modern world. Excessive fat storage, leading to obesity, is the default situation unless specific action is taken (Lobstein, Baur, & Uauy, 2004).

A range of experimental and epidemiologic studies have demonstrated that a sedentary lifestyle is a significant risk factor for numerous hypokinetic diseases (for example, cardiovascular disease, hypertension, obesity, type 2 diabetes), with increased prevalence occurring at advancing age in both sexes. In contrast, an active lifestyle offers considerable protection against several diseases with a suggestive dose-dependent relationship (Bouchard, 1997). However, there is no consensus in the scientific community regarding this relationship. Rowland (2001), Twisk (2001) and Biddle et al. (2004) after critical literature analysis, indicate that there is only marginal evidence that physical activity is beneficial for health and there is hardly any indication that these health benefits have a threshold value. Physical activity may have a positive influence on reducing cardiovascular risk in youth, although the evidence to date is unconvincing, and many key research questions have not been adequately tested. Biddle et al. (2004) have suggested that the lack of association between physical activity and CVD risk may be due to a difficulty in accurately measuring physical activity.

In contrast, a study by Katzmarzyk et al. (2001) outlined the contribution of physical fitness and obesity as the future risk predictors of coronary heart disease, based on the Framingham Heart Study risk factor categories. The results of this study indicate that body fat as much as cardiorespiratory fitness is an important determinant of the coronary heart disease risk derived from established risk factors. Physical activity can have a significant effect on public health, particularly due to its influence on cardiovascular diseases (Pate et al., 1995). The clinical manifestation of these diseases is being seen at progressively earlier ages in the lifespan, including childhood and adolescence. Further, it is widely recognised that health outcomes in adulthood are related to behavioral patterns adopted during childhood (WHO, 1990). Therefore, strategies for the effective prevention of chronic disease must be based on lifestyle changes, mainly in youngsters. The lifestyle parameter of highest interest in chronic disease prevention, treatment and management is physical activity (Blair, Kohl, Barlow, Paffenbarger, Gibbons, & Macera, 1995).

In obese Latin-American children, the disorders related to lifestyle parallel those observed in children from the United States and Canada (Confederation, 1998). Adverse and immediate health problems associated with excess body weight are psychological dysfunction and social isolation in children who are overweight or obese (Friedman, Wilfley, Pike, Striegel-Moore, & Rodin, 1995; Must, 1996; Must & Strauss, 1999). However, the association between physical activity or sedentary behaviour and obesity in children and adolescents has not been consistently shown (Ekelund et al., 2001; Deforche, De Bourdeaudhuij, Debode, Vinaimont, Hills, Verstraete, & Bouckaert, 2003; Tremblay & Willms, 2003).

Recently, Ekelund et al. (2004a; 2005) indicated that physical activity explains only a small amount of the variation in body fatness. On the other hand, Maffeis (2005) stated that this conclusion apparently reduces the relevance of the intensity of physical activity in the maintenance of childhood obesity and seems to frustrate the reasonable expectancy for the role potentially played by exercise and physical activity both in the prevention and treatment of overweight. Ekelund et al. (2005) indicated that it is disappointing that the results for physical activity do not explain a larger proportion of the variance in body fatness.

Styne (2005) suggested that there is no risk associated with a decrease in sedentary activity or a reasonable and sustainable increase in physical activity, and there is benefit in terms of improved insulin sensitivity, among other factors. Thus, even if we have difficulty in directly and substantially linking physical activity to fat mass, the risk-benefit ratio is so high that there is no justification for not supporting an increasingly active lifestyle.

Despite the lack of a consistent relationship between physical activity behaviour and obesity, rates of childhood obesity are increasing worldwide with around ten per cent of the world's school-aged children estimated to be carrying excess body fat. To provide some examples, in China, one in 13 children is overweight, in Brazil one in seven, in Italy one in three and in Australia one in five. (Lobstein et al., 2004).

An important concern regarding the prevalence of overweight was recently outlined by Jolliffe (2004). This analysis showed that not only have more children become overweight in the last three decades in the United States, but those who are overweight have been getting steadily more overweight and progressively heavier. Burke et al. (2005) using the BMI as a predictor in a longitudinal survey of a cohort of Australian children followed from the 16th week of gestation to 8 years of age showed that the increasing rate of overweight including obesity, is associated with an increase in cardiovascular risk factors very early in life.

Freedman et al. (2004) verified that BMI reflects the positive association between height and adiposity among children, and also height related to adult adiposity. Recently, in a study to verify the utility of childhood BMI in the prediction of adulthood disease, Janssen et al. (2005) showed that overweight and obesity during childhood, as determined by both the Center for Disease Control and Prevention (CDC) and International Obesity Task Force (IOTF), BMI cut-off points are strong predictors of obesity and coronary heart disease risk factors in young adulthood. It is interesting to note that differences in the predictive capacity between both suggested cut-off points are minimal.

Obesity is not solely the result of low levels of physical activity but rather arises from an energy imbalance in which energy intake exceeds energy expenditure (Hill, 2004). Viewed from an energy balance perspective, obesity can be thought of as a failure of our biological regulatory system to balance the energy we take from the food and drink consumed with the energy expended as metabolism and to perform physical activity. While some have approached obesity as primarily a result of a 'defect' in the biological system itself, the problem may also be viewed as related to an evolving environment that is overwhelming the biological regulation balance and leading to obesity (Astrup, O'Hill, & Rossner, 2004).

Data from randomised trials to support any preferred strategy to achieve weight control in children and adolescents, or to prevent the development of overweight, are currently lacking (Batch & Baur, 2005; Dietz & Robinson, 2005).

Traditionally, physical activity epidemiologists have been primarily concerned with quantifying activity, whereas the quantification of inactivity is often simply inferred from the absence of active pursuits. However, the opportunities for many individuals to be active are fast disappearing and inactivity may exert effects on health which are independent of the effects of activity (Dietz, 1996). Arluk et al. (2003) found a strong correlation between obesity status and time that children spend in inactive behaviour patterns. In the same way, Fleming-Moran and Thiagarajah (2005) showed that TV exposure independently increases odds of overweight by 50% for both genders, when other covariates are controlled. Efforts to accurately characterise inactivity merits much more attention than has been the case to date (Livingstone et al., 2003).

The WHO Global Strategy on Diet, Physical Activity and Health (WHO, 2004, p. 8) provides the following statement in relation to physical activity:

> “Physical activity is a key determinant of energy expenditure, and thus is fundamental to energy balance and weight control. Physical activity reduces risk for cardiovascular diseases and diabetes and has substantial benefits for many conditions, not only those associated with obesity. The beneficial effects of physical activity on the metabolic syndrome are mediated by mechanisms beyond controlling excess body weight. For example, physical activity reduces blood pressure, improves the level of high density lipoprotein cholesterol, improves control of blood glucose in overweight people, even without significant weight loss, and reduces the risk for colon cancer and breast cancer among women.”

Children are not small adults and extrapolation from adult research to children and adolescents should be undertaken with caution (Steinbeck & Pietrobelli, 2005). The point at which excess fat becomes pathologic and requires intervention is still subject to debate and is even more complicated in the paediatric population where ongoing growth and development is occurring. Nevertheless, there is increasing evidence that adverse outcomes related to obesity are increasing in both children and adults (Daniels, 2001).

Kortelainen (1997) evaluating the autopsies of 210 children aged 5-15 years found that the ponderal index (body weight/height^3) was a significant predictor of heart weight and the presence of coronary artery intimal fatty streaks. Fatty streaks in coronary vessels were not found in the leanest individuals. The American Heart Association (Kavey, Daniels, Lauer, Atkins, Hayman, & Taubert, 2003) stated that convincing evidence has emerged that links defined risk factors in adults with an accelerated atherosclerotic process. Pathological data have shown that atherosclerosis begins in childhood and that the extent of atherosclerotic change in children and young adults can be correlated with the presence of the same risk factors identified in adults. It has been suggested that it is highly desirable to initiate healthful lifestyle training in childhood to promote improved cardiovascular health in adult life.

Recently, Fulton et al. (2004) in an interesting review, described public health and clinical recommendations for physical activity and physical fitness for youth and assessed whether these recommendations specifically addressed overweight youth. These authors identified ten organisations for a total of 13 public health physical activity or physical fitness recommendations for youth. This paper revealed three major findings. First, physical activity recommendations written for the public health community tended to include specific components of physical activity prescription whereas recommendations for physical fitness did not include specific components of physical fitness. Second, recommendations for clinical assessment of physical activity and physical fitness were rare or vague. Clinical recommendations to counsel about physical activity were explicitly described by nearly half of the organizations, however, explicit counselling recommendations for physical fitness were rare. Third, public health physical activity and physical fitness recommendations did not generally address overweight youth.

From the same perspective, Hills and Byrne (2004) indicated that the public health messages about exercise have focussed on improvements in general health and fitness rather than on weight loss, prevention of weight gain or weight regain, and added that about 2.5 times more exercise than the US Surgeon General's recommendation is needed to maintain energy balance and thus a defined body weight.

In a public health overview, Evans et al. (2005) demonstrated that there is strong public support for interventions aimed at reducing overweight and obesity among children and adolescents. It is interesting to note that in general more educated survey respondents are supportive of the interventions. However, those respondents with greater incomes and those with children at home were less likely to support weight evaluation in schools. This contradictory situation has been referenced by Baur (2005) who said that it is ironic that such an obvious medical condition in children can apparently be almost invisible to the parents of such children. This illustration of parents' lack of acknowledgment of their child's weight status has also been verified in other recent studies (Carnell, Edwards, Croker, Boniface, & Wardle, 2005; Jeffery, Voss, Metcalf, Alba, & Wilkin, 2005).

The notion that regular exercise may prevent or delay the onset or progression of disease is not new. Two developments in modern life have increased interest in this area as a topic for investigation. Firstly, advances in technology have altered most of our occupations and modes of transportation such that many people now expend less energy in these daily activities. Therefore, if a sedentary life contributes to ill health, the evidence should now be more pronounced than ever before. Secondly, chronic degenerative diseases have replaced many infectious and contagious diseases as causes of death and disability (Montoye, 2000).

A relatively new phenomenon in developing countries such as Brazil is the combination of underweight in children and overweight in adults, frequently coexisting in the same family (Caballero, 2005). For example in Sao Paulo, Brazil, 30% of children living in shantytowns are malnourished (Sawaya, Dallal, Solymos, de Sousa, Ventura, Roberts, & Sigulem, 1995). Traditionally, obesity has been linked with abundance (Caballero, 2005), but Drewnowski and Specter (2004) provide a good picture of the present situation saying '…people are not poor by choice and they become obese primarily because they are poor.' Solutions to the obesity epidemic will in part require that healthy food becomes accessible and affordable, and that our knowledge about energy expenditure and physical activity characteristics can be improved.

To better illustrate the necessity for applicable techniques to measure energy expenditure to quantify diet and physical activity, a number of comparisons between developed and developing countries are pertinent. The following example is based on data from Australia and Brazil. First, Australia has a population of 20,370,000 (ABS, 2005) while Brazil has a population of 185,000,000 (Brazilian Institute of Geographic Statistics (2005). Secondly, the percentage of overweight and obesity in the Australian population is around 60% (12,222,000) (ASSO, 2005) whereas in the Brazilian population, the percentage is 40.6% (75,110,000). However, 10% of the Brazilian population is also undernourished (18,500,000). Third, the percentage of the population under 14 years of age in Australia is 20.7% (4,216,290) whereas in Brazil, 36% (66,600,000) of the population is under 14 years of age. Finally, among Australian children the percentage of overweight and obesity is 25% (1,054,148) whereas among Brazilian children the percentage is 14% (9,324,000) and the percentage of undernourished children is 8.6% (5,727,600). In summary, Brazil has around 10 times more overweight and obese children than Australia. Considering the escalating global prevalence of obesity and the negative influence of excess weight early in the life on adult obesity, Brazilian health professionals have an almost insurmountable challenge.

Knowledge of walking energy expenditure and physical activity patterns in children is important for three main reasons. First, the physiological cost of an activity such as walking is an important determinant of functional capacity and physical fitness, and it contributes to fatigue rate in physical activity. Secondly, the energy equivalence and intensity of the physical activity are important elements in relation to the exercise prescription. If the energy expenditure and the exercise intensity differ among children and adults, individualised prescriptions should vary appropriately. Third, walking is a natural, accessible and safe physical activity and does not require special ability or equipment. For most individuals walking is the key activity of daily life and has significant potential to increase the daily total energy expenditure.

The journey to school is a potentially important opportunity for establishing daily physical activity and many schemes have been introduced to promote active transport to and from school (Rowland, DiGuiseppi, Gross, Afolabi, & Roberts, 2003). Despite the enthusiasm for such approaches, there is little evidence for the magnitude of the contribution that active commuting to school might make to children's overall physical activity (Tudor-Locke, Ainsworth, Whitt, Thompson, Addy, & Jones, 2001). Amorim et al. (2006) found an inverse correlation between BMI and walking MET minutes/week (3.3 * walking minutes * walking days) in 14 year-old, overweight and underweight Brazilian school-aged girls and showed that overweight girls do not walk less than their leaner counterparts. These results can be explained if one considers the social context of participants and that walking commonly represents the largest component of daily physical activity, mainly as a means of transportation. However a limitation in this study was the use of a questionnaire, an indirect technique.

Despite the existence of a number of approaches to increase walking and biking to school, very few have been evaluated with respect to their effect on physical activity patterns. Up to now, no internationally accepted standards for the measurement of physical activity in children and no global estimates of children's physical activities exist. Devices such as heart rate monitors, pedometers and accelerometers are becoming increasingly popular as objective measurement tools for physical activity. These devices reduce the subjectivity inherent in survey methods and can be used with large groups of individuals (UNECE-WHO, 2004). The preferred method to determine energy expenditure is likely to principally depend on factors such as the number of individuals to be monitored, the time period of measurements and the finances available (Ainslie, Reilly, & Westerterp, 2003).

While the optimum epidemiological tool for assessing both total energy expenditure and associated patterns of physical activity does not exist, those who work in the area have drawn up an ambitiously long 'wish' list criteria for the 'ideal' method. The ideal method should be accurate, precise, objective, simple to use, robust, cause minimal intrusion into habitual physical activity patterns, socially acceptable, time-efficient and it should allow continuous and detailed recording of usual activity patterns (Livingstone, 1997).

Research questions

In this thesis, the main goal was to identify a suitable indirect and objective measurement technique for energy expenditure and physical activity patterns in children. The technique should be reproducible and validated against a direct measurement method, be inexpensive and applicable to large cohorts. To achieve the main goal it was necessary to conduct four sequential studies. Research questions for each study are outlined below:

Chapter II

A – Does equipment used for airflow collection during indirect calorimetry influence RMR measurements in children?

B – What is the effect of TV viewing on RMR measurement in children?

C – Does body position influence RMR measurement in children?

Chapter III

A - Is the self-selected speed (SS) determined using an indoor over-ground protocol reproducible on the treadmill?

B - Is a 10 min familiarisation protocol based on SS sufficient to achieve reliable oxygen uptake measures between tests?

C - Is submaximal $\dot{V}O_2$ reliable within- and between-days in children?

D – Is heart rate (HR) measurement reliable within- and between-days in children performing submaximal exercise?

E - Is the preferred pace the most economical walking speed in children?

F – Is the gross economy of walking reliable within- and between-days in children?

G – Is the SS an adequate exercise intensity to increase fat oxidation in children?

H – Is the substrate oxidation pattern reproducible in children performing submaximal exercise?

I – Are there differences in oxygen uptake between girls and women performing submaximal exercise at intensities based on SS?

J - Are there differences in substrate oxidation pattern between girls and women performing submaximal exercise at intensities based on SS?

Chapter IV

A – Are the determination of upper body or lower body activity predominance and the utilisation of selected HR equations capable of improving the accuracy of free-living energy expenditure (EE) measurements in children?

B – Is the simultaneous use of accelerometer and HR analysed using branched equations capable of improving the accuracy of free-living EE measurements in children?

C – What are the within- and between-subject variability in mean daily physical activity (MDPA) and total energy expenditure (TEE) from 7 days monitoring using accelerometer output and HR equations?

D - Are there differences in TEE between weekdays and weekends in children?

E – Are there influences of different combinations of weekdays and weekend days on MDPA and TEE reliability?

F – Are there specific time windows during school days able to be represent children's physical activity pattern?

Chapter V

A – Is the standard metabolic equivalent (MET) value applicable to children?

B - What is the error magnitude in a range of walking and running speeds between measured and standard MET in children?

C – Are there differences in minutes expended at moderate-to-vigorous physical activity (MVPA) from measured and standard MET quantified from 7 days of free-living monitoring?

D - Are there differences in physical activity pattern between weekdays and weekends in children?

E – Do the most commonly utilised HR indices to measure physical activity intensity explain physical activity patterns in children?

F – Are there differences in the output of HR indices when classifying children using the current physical activity recommendations?

Chapter II – RESTING METABOLIC RATE IN CHILDREN

2.1 Literature review

Basal metabolism as defined by Durnin and Passmore (1967), is the minimum amount of energy expenditure compatible with life, or the energy expenditure of an individual at complete rest in a fasted state. The basal metabolic rate (BMR) is responsible for approximately 60-75% of the total daily energy expenditure in humans (Poehlman, 1989; Sharp, Reed, Sun, Abumrad, & Hill, 1992) and is usually measured under resting conditions by indirect calorimetry using measures of respiratory gas-exchange.

BMR is ideally measured as soon as the individual wakes up in the morning, having been in a fasted state for 10-12 hours. The individual should be completely relaxed and lying in a supine position in a thermo-neutral environment (Durnin, 1996). Methodological difficulties associated with the attainment of these ideal conditions has led to the use of the resting metabolic rate (RMR), a procedure which requires an interval of only four hours following a light meal and a relatively short measurement time of between 30 and 60 minutes.

Studies using whole body room calorimeters have reported excellent reproducibility of energy metabolism in 24-hour periods, when food ingestion can be quantified and physical activities measured with margins of error as low as 2% (James & Schofield, 1990).

To ensure greater confidence in indirect calorimetry measurements, careful calibration of flow sensors and gas analysers at regular intervals and before each measurement should be undertaken, and standard conditions for testing should be adhered to. These include length of fasting and rest period, achieving steady state, and assessment of data for physiological validity (Reeves et al., 2004).

Gallagher et al. (1998) showed that BMR estimated from tissues and organ masses and their corresponding metabolic rates, were not significantly different from BMR measured by indirect calorimetry. These studies showed a strong association between

BMR and organ weights because of their high metabolic rates (Elia, 1992). A variety of factors influence RMR, however according to Arciero et al. (1993), the primary determinant is the fat free mass (FFM) that reduces by approximately 2-3% per decade in women and men, respectively. Women tend to have a significantly lower RMR at all the ages as a result of their reduced FFM (Weinsier, Schutz, & Bracco, 1992). The tissues and organs that comprise the FFM contribute to further diversity of metabolic activity. The extracellular mass has low metabolic activity, while the cellular mass, which corresponds to between 50 and 60% of the FFM, is responsible for the largest portion of metabolism and is composed of the viscera, brain, blood and muscle mass (Ellis, 1996). The majority of the RMR of the body (approximately 60%), is associated with organs such as the liver, kidney, heart, and brain, which account for only about 5-6% of total body weight. Although skeletal muscle is the largest composite tissue of the body, accounting for about 40% of body weight, its estimated RMR is low (~10-15 $\mathrm{kcal.kg^{-1}.d^{-1}}$), so its contribution to the total energy expenditure of the body is about 20-25% (Elia, 1992).

Other factors that influence the RMR are sex and age, with lesser values in women and in older individuals. The relationship between age, sex and RMR is partially explained by the absolute values of the FFM (Arciero et al., 1993), where a lower weight will necessarily demand reduced energy expenditure. Measurement of RMR and total daily energy expenditure has improved our understanding of the pathophysiology of human obesity and has the potential to improve the management of obesity in children (Goran, 2000).

Pediatric RMR prediction equations have been developed using variables such as age, stature, and body mass. However, only 52 to 89% of the variability in RMR is explained by these equations (Henry et al., 1999). It is recommended that RMR be measured in children whenever possible to reduce the risk of misclassification and errors regarding estimation of energy intake and balance (Finan et al., 1997; Verhoeven et al., 1998; Nieman et al., 2005).

Maffeis et al. (1993) confirmed the associations in childhood between RMR and body composition previously reported in adults and adolescents. FFM, the tissue with the highest metabolic activity, was the best predictor of RMR in both boys and girls.

The values for RMR per kilogram of FFM are dependent on both the relative composition of the FFM, muscle and non-muscle tissue, and the metabolic activity of the various organs and tissues constituting the lean body mass.

Maffeis et al. (1990) also showed that if corrected for kilogram of FFM, the anthropometric variable which accounts for the major variations of RMR (75%), RMR in obese subjects was lower than in normal weight controls. Stensel et al. (2001) argued that when body composition is appropriately controlled for, RMR does not differ significantly between obese and non-obese boys.

Lazzer et al. (2005), assessing the impact of a weight reduction program on body composition and energy expenditure in obese adolescents, showed that BMR, sleeping, sedentary activity and daily energy expenditure decreased significantly, even after adjustment for FFM, probably due to reductions of organ mass and metabolic rates. In addition, the energy cost of physical activities decreased. These authors concluded that these changes in energy metabolism after a weight reduction period might explain the frequent weight regain of post-obese subjects.

Ethnic differences in RMR during puberty were studied by Sun et al. (2001). The results of this study indicated that African-American children had lower RMR than white children after adjustment for fat mass, lean mass and pubertal stage. Another interesting observation was that RMR decreased as puberty progresses, after adjustment for ethnicity, sex, fat mass and lean mass. Yanovski (2001) suggested that the magnitude of pubertal effect for children at Tanner stage 3 and 4 exceeds the difference in RMR they found between African-American and white children.

Among studies that have examined the RMR relationship with pubertal maturation, the findings are inconsistent. Most of these studies have been cross-sectional and used Tanner staging to gauge pubertal maturation, and many have presented combined results for boys and girls. Spadano et al. (2004) studied longitudinally the pattern of change in RMR relative to menarche, a well-defined event in the course of pubertal maturation for females. The results of this study suggested an elevation in RMR around the time of menarche, based on the comparison of absolute RMR, FFM and fat mass between menarche and 4 year after. Another study by the same group

(Spadano, Bandini, Must, Dallal, & Dietz, 2005) suggested that the observed elevation in RMR is not specific to menarche but most likely begins in mid-puberty and persists through menarche. These authors suggest that the lack of significance of the pubertal status variable in the adjusted RMR model may reflect the paucity of RMR measures around menarche as well as the study's limited power.

One possible explanation given by Spadano and colleagues was that peak height velocity almost always precedes menarche and usually occurs in girls around Tanner stage 3, and an increase in the growth velocity of the more metabolically-active organs may contribute to an elevation in RMR around the time of menarche. Changes in growth hormone (GH) during puberty in girls provides one plausible mechanism for the observed elevation in metabolic rate (Gregory, Greene, Jung, Scrimgeour, & Rennie, 1991). Elevations in GH during puberty may also be responsible for pubertal insulin resistance, which in turn may contribute to the elevation in metabolic rate around the time of menarche (Amiel, Sherwin, Simonson, Lauritano, & Tamborlane, 1986).

The presently available RMR-body composition models are based on two fundamental concepts: 1) that only metabolically-active components contribute to RMR; and 2) that there are quantitative and measurable associations between RMR and metabolically-active components. All metabolically-active components can be organised according to the five-level model, which indicates that the ~40 body components are distributed into five distinct but connected levels: atomic, molecular, cellular, tissue/organ, and whole body (Wang, Heshka, Gallagher, Boozer, Kotler, & Heymsfield, 2000).

Individual tissues and organs can be divided into two groups, one with high RMR (e.g., brain, heart, liver and kidney) and the other with low metabolic rates (e.g., skeletal muscle, skeleton and adipose tissue) (Wang et al., 2000). The same group of authors suggested that the tissue/organ components associated with FFM vary relative to FFM as a function of body size and FFM (Heymsfield, Gallagher, Kotler, Wang, Allison, & Heshka, 2002). However, Bosy-Westphal et al. (2004) argue that, for practical purposes in humans, there is no decrease in the organ metabolic rate with increasing organ mass, which has been deduced from interspecies comparison.

Results from the former study indicate that at the cellular level, RMR seems to be constant with increasing organ mass and inter-individual RMR differences in under- or overweight are more reflection of an altered ratio of tissue mass to body mass.

The decline in RMR during growth may be due to changes in body composition or to changes in the metabolic rate of individual organs and tissues. Hsu et al. (2003) confirmed the hypothesis that the proportion of FFM in high metabolic rate organs, specifically, liver and brain, is greater in children than in young adults. However, after this disproportion was accounted for, the specific organ and tissue metabolic constants available in the literature (Elia, 1992) were not adequate to account for RMR in children. These results imply that the decline in RMR per kilogram body weight (or per kilogram FFM) during the growing years is likely due to both changes in body composition and changes in the metabolic rate of individuals' organs and tissues.

The relative difficulties of taking direct measures of RMR make it an impractical measure in large populations, or potentially for health professionals such as nutritionists and physicians. A range of prediction equations for the assessment of RMR have been published, the most widely used being those of Schofield (1985) and FAO/WHO/UNU (1985). However, equations need to be used with caution as they may over- or underestimate RMR in different groups.

Firouzbakhsh et al. (1993) verified that when compared in specific age groups, the Schofield equations provide the best estimates for RMR measurements in children who are within 90-110% of their ideal body weight and suggested that both Schofield (1985) and FAO/WHO/UNU (1985) equations are reliable estimates of the metabolic rate in healthy children. On the other hand, Rodriguez et al. (2002) assessed the degree of agreement between indirect calorimetry and five equations commonly used to predict RMR in obese and non-obese children and adolescents. Based on their results these authors recommend the use the 'Schofield-Height and Weight' equation in field studies with a mixed population of obese and non-obese children and adolescents; however the 'FAO/WHO/UNU equation' may be useful in girls and 'Schofield-Weight equation' in non-obese children.

Schmelzle et al. (2004) compared the accuracy and precision of RMR predicted in obese children using previously published equations in which DXA-derived body composition measurements were used to predict RMR. These authors concluded that RMR in obese children should not be predicted from equations derived from an adult or non-obese population, and suggested a new equation including DXA measurements. However, Flodmark (2004) criticised Schmelzle's paper considering that measurements made by DXA can be significantly affected by bone maturation, age, sex, tissue thickness, skeletal content of FFM, choice of instrumentation and choice of software. In addition, the inaccuracy of DXA for children, especially when an individual is examined, might influence the accuracy of the established equation.

Derumeaux-Burel et al. (2004) developed equations to predict RMR in a large population of obese children from 3 to 18 years of age. It is important to consider the author's recommendation that these equations are used in a period of body weight stability because body weight changes are associated with modifications in the relation between lean mass and metabolic rate.

Recently, Muller et al. (2004) considering that the current standard references for RMR are based on measurements made in the first part of the past century in various races and locations, investigated the application of the WHO equations in healthy subjects living in a modern, affluent society in Germany. This study showed that RMR prediction by the WHO formulae systematically over- and underestimated RMR and that RMR prediction from a weight group-specific formula is recommended in underweight subjects.

2.2 Combined effects of body position, apparatus and distraction on resting metabolic rate in children. Methodological issues

Modified from: 'Amorim PRS, Byrne NM and Hills AP (2007). Combined effect of body position, apparatus and distraction on children's resting metabolic rate.' International Journal of Pediatric Obesity, 2 (4), 249-256, 2007.

2.2.1 Introduction

There is worldwide consensus that obesity in childhood is a significant health problem related to increased paediatric and adult morbidity and mortality (Must, 1996; Must & Strauss, 1999). Despite the proposition that the impact of obesity on mortality has decreased over time (Flegal, Graubard, Williamson, & Gail, 2005), a recent study has showed that adiposity in adolescence has been associated with premature death in younger and middle-aged women, with an increased premature mortality rate even for moderately increased adolescent body mass index (BMI) (van Dam, Willett, Manson, & Hu, 2006). It is further recognised that not only have more children become overweight in the last three decades but overweight children have been getting heavier (Jolliffe, 2004).

Diagnosis and subsequent treatment of childhood obesity is therefore necessary and knowledge of an individual child's energy needs is essential to develop an appropriate diet and physical activity prescription. Measurement of resting metabolic rate (RMR) and total daily energy expenditure has improved our understanding of the pathophysiology of human obesity and also has the potential to improve the management of obesity in children (Goran, 2000).

Paediatric RMR prediction equations have been developed using variables such as age, stature, and body mass. However, only 52 to 89% of the variability in RMR is explained by these equations (Henry, Dyer, & Ghusain-Choueiri, 1999). It is recommended that RMR be measured in children whenever possible to reduce the risk of misclassification and errors regarding estimation of energy intake and balance (Finan, Larson, & Goran, 1997; Verhoeven, Hazelzet, van der Voort, & Joosten, 1998; Nieman, Austin, Chilcote, & Benezra, 2005).

RMR is the major component of total energy expenditure in children, especially when a sedentary lifestyle is dominant (Flodmark, 2004). Measurement of RMR by indirect calorimetry allows the measurement of the amount of oxygen consumed ($\dot{V}O_2$) and carbon dioxide (CO_2) produced during a specific period of time. These values can be converted to calories and an estimate of energy expenditure per day gained. To ensure greater confidence in indirect calorimetry measurements, careful calibration of flow sensors and gas analysers at regular intervals, and before each measurement, should be undertaken, and standard conditions for testing should be adhered to. These 'standard conditions' include length of fasting and rest period before measurement, achieving steady state, and assessment of data for physiological validity (Reeves, Davies, Bauer, & Battistutta, 2004). Such assessment, particularly for children, is very tedious as it is imperative that children rest quietly over an extended period of time under the same conditions to gain reliable results.

Sampling expired air can utilise a mouthpiece and nose-clip, facemask or ventilated hood however comparative data derived from these three techniques has been inconclusive. Some studies in adults have found no difference between hood and mask systems (McAnena, Harvey, Katzeff, & Daly, 1986), or among ventilated hood, facemask and mouthpiece (Segal, 1987). Others had verified reproducible metabolic data for facemask and ventilated hood, however decreased reliability for mouthpiece as test duration increased (Isbell, Klesges, Meyers, & Klesges, 1991) or that both, mouthpiece and facemask increase the oxygen consumption and the energy expenditure, indicating that there are differences with the gas measurements depending on the apparatus used (Forse, 1993; Roffey, Byrne, & Hills, 2006).

An accurate measure of RMR is very important in the determination of energy needs. Understanding the combined effect of some variables on the variability in RMR measurements should help in the development of more accurate protocols to measure RMR in children. However, no study to date has compared the impact of different apparatus, as well as body position, on children's RMR measurements.

We hypothesised that different combinations of three methodological parameters – breathing apparatus, body position and distraction would explain the differences in RMR measures reported between studies. The purpose of the present investigation was to verify the combined effect of body position (sitting or lying), apparatus (mouthpiece and nose-clip or facemask) and distraction (TV viewing or no TV viewing) on children's RMR.

2.2.2 Materials and methods

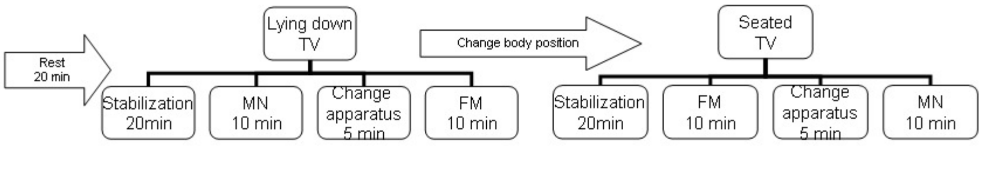
2.2.2.1 Participants

Experiments were carried out on 14 children aged 8-12 (mean age = 10.1±1.4 yr, mean mass = 34.5±6.5 kg, mean height = 144±10.2 cm) recruited from the Brisbane metropolitan area through advertisements distributed in schools. A *post hoc* analysis indicated a statistical power of 0.86 at the 0.05 significance level (G*Power 3.0.1 for Windows, 2007). Parents or caregivers of respondents were interviewed by telephone and children were classified as eligible based on age and medical history. Exclusion criteria included individuals with known medical conditions such as type I or type II diabetes mellitus or any metabolic disease. Eligibility for the study was dependent on participant's ability to tolerate any necessary instrument or apparatus used during testing sessions. All participants were in good health and were not taking medications known to influence metabolic rate. Participants and their parents or caregivers gave written informed consent that followed the guidelines of the Queensland University of Technology Human Research Ethics Committee.

2.2.2.2 Study design

Each participant underwent 2 test sessions, one week apart under three different situations: a) using mouthpiece and nose-clip (MN) or facemask (FM); b) sitting or lying and c) TV viewing, using an appropriate movie for the age and stage of development of children or no TV viewing. Sessions 1 and 2 follow the same design shown below (Figure 2.1). However in session 2 there was no TV viewing and apparatus order was changed.

Figure 2.1 – Study design



Participation in morning or afternoon sessions was randomised among participants. Morning sessions were conducted between 7:00 and 8:30 am, and afternoon sessions were scheduled between 3:30 and 5:00 pm. For the morning session participants were instructed to remain in an overnight fasted state (no food or drink, except for water) and for afternoon sessions participants were instructed to finish their lunch at least 4 hrs before the scheduled session time. When participants had not complied with the required 4-hours dietary restriction, measurements were not performed and testing was rescheduled. A criterion for afternoon measurements was to have an RQ of 0.85 or less. For both sessions, participants were instructed to abstain from moderate and strenuous exercise in the previous 24 hours.

2.2.2.3 Data Collection

On the morning or afternoon of testing participants arrived at the exercise physiology laboratory by car having been instructed to minimise physical activity prior to arrival. Prior to RMR measurement, anthropometric measures were taken using standard protocols with the participant wearing comfortable clothes and without shoes.

Data collection took place in a thermo-regulated environment. Participants were fitted with a nose and mouth paediatric face mask (FM) (series model 8950 - Hans Rudolph, Inc.) with a two-way breathing T-shaped valve and pneumotach. To improve the seal between face and mask an Ultimate Seal^{TM} gel was used and tested for leakage by passing a mirror around the edges of the mask while the participants breathed. Leakage was eliminated by readjustment of the mask. On the alternate test session, participants were fitted with a silicone mouthpiece (standard type - Hans Rudolph, Inc.) and a disposable nose-clip (MN) (model 9014 - plastic and foam - Hans Rudolph, Inc.). Participants were also fitted with a Polar transmitter (Polar WearLink Transmitter, Polar Electro Oy, Finland) and a receiver model S610 (Polar Electro Oy, Finland). As a further indication of resting state, an accelerometer (RT3-Stayhealthy, Inc., Elkader, IA) was attached to a waist belt on the right hip.

Participants were rested for 20 min and monitored periodically to ensure that they remained awake. Any movement or any interference in normal breathing (e.g. cough) observed during the measurement was recorded. Ventilation (V_E), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) were derived from breath-by-breath samples using a MOXUS Modular $\dot{V}O_2$ System. The respiratory quotient (RQ) was calculated as the ratio of $\dot{V}CO_2$ relative to $\dot{V}O_2$. A turbine attached to the inspired side of a Hans Rudolph T-shaped valve measured volume and airflow, and the device was calibrated before each measurement using a certified 3L syringe. Verification is achieved when measured stroke volume at 60 L/min, 90 L/min and 120 L/min is within ±1.5% of syringe volume. The gas analysers were also calibrated before each measurement using known standard gas concentrations. Calibration was complete when gas analysers measured oxygen and carbon dioxide concentrations within ±0.2% and ±0.08% of expected values, respectively.

2.2.2.4 Data Analysis

Any abnormal values were checked by notes previously taken of any movement observed during the measurement together with the RT3 data results and, when matched, these records were excluded from analysis. Data were first analysed considering four measurements for each methodological parameter, body position, apparatus and distraction. Following this procedure data were analysed according to the eight combinations of parameter situations, as follows: 1) watching TV + mouthpiece + lying (TVMNLY); 2) watching TV + facemask + lying (TVFMLY); 3) watching TV + mouthpiece + seated (TVMNSEAT); 4) watching TV + facemask + seated (TVFMSEAT); 5) mouthpiece + lying (MNLY); 6) facemask + lying (FMLY); 7) mouthpiece + seated (MNSEAT) and 8) facemask + seated (FMSEAT).
All statistical analyses were carried out using 5 min steady state data, depending on the lowest coefficient of variation (CV) between minutes 1 to 5, 2 to 6, 3 to 7, 4 to 8, 5 to 9 and 6 to 10 for each measured situation, after the stabilization period. RMR was calculated according to the Weir equation (Weir, 1949).

Data were presented as mean values and standard deviations (SD). The coefficient of variation (CV) was used to compare within- and between-tests RMR variability for all situations and Paired T-test was used to verify differences between CVs. Bland and Altman analysis (Bland & Altman, 1986) was used to verify agreement and relative bias for RMR between body position (lying X seated), apparatus (face mask X mouthpiece) and distraction (TV X no TV). Paired T-tests were used to compare morning and afternoon measurements between-subjects. Repeated measures ANOVA were used to verify the significance of differences in mean $\dot{V}O_2$, $\dot{V}CO_2$, RQ, RMR, HR and RT3 counts values for the eight cited combinations. When significant *F*-ratios were obtained, Bonferroni *post-hoc* tests were performed to locate differences among means. Agreement and relative bias for $\dot{V}O_2$, $\dot{V}CO_2$, RQ and RMR were assessed by the Bland and Altman method (1986). Values were considered significant at $P<0.05$. Statistical analyses were carried out with SPSS for Windows (version 13.0, 2004, SPSS, Chicago, IL, USA).

2.2.3 Results

The mean RMR CV within- and between-tests for each of the eight combined parameter situations is presented in Table 2.1. Paired T-test indicated mean RMR CV within-test for apparatus (p=0.048) and for distraction (p=0.043) was statistically significant different. Average time for the lowest CV was observed between the third and seventh minutes of measurement after the stabilization period for each measured parameter.

Table 2.1 – RMR CV within- and between tests for each situation in percentages

	Body Position		Apparatus		Distraction			
	MN	FM	Lying	Seated	MNly	MNseat	FMly	FMseat
CV W	7.0	7.0	4.0	7.0¥	6.0	8.0	5.0	9.0*
CV B	12.0	13.0	13.0	13.0	10.0	10.0	13.0	12.0

CV W = within test; CV B = between test; MN = mouthpiece and nose-clip; FM = face mask

¥ Statistically significant difference ($p<0.05$) from Lying

* Statistically significant difference ($p<0.05$) from FMly

Descriptive statistics of RMR values for body position, apparatus and distraction is showed in Table 2.2. The lowest mean value *per* parameter was using facemask (1114.5±55.6 kcal.d^{-1}), lying down (1128.2±38.3 kcal.d^{-1}) and watching TV (1135.0±57.1 kcal.d-^{1}).

Table 2.2 - RMR descriptive statistics for apparatus, body position and distraction

RMR	Apparatus		Body Position		Distraction	
(kcal.d^{-1})	MN	FM	Lying	Seated	TV	No TV
Mean	1185.5	1114.5	1128.2	1171.7	1135.0	1189.0
SD	39.5	55.6	38.3	72.7	57.1	38.0
Minimum	1146.0	1066.0	1072.0	1066.0	1087.0	1146.0
Maximum	1222.0	1182.0	1157.0	1171.7	1213.0	1236.0

MN = mouthpiece and nose-clip; FM = face mask

Biases from Bland and Altman analysis are showed in Table 2.3 for RMR between body position (lying X seated), apparatus (face mask X mouthpiece) and distraction (TV X no TV). Statistically significant difference was observed in distraction analyses for FMSEAT watching TV from no watching TV considering $\dot{V}O_2$ (-16±13 ml.min^{-1}), $\dot{V}CO_2$ (-14±22 ml.min^{-1}) and for RMR (-110±152 kcal.d.$^{-1}$).

Table 2.3 – Bias for $\dot{V}O_2$ (ml.min^{-1}), $\dot{V}CO_2$ (ml.min^{-1}), RQ and RMR (kcal.d^{-1})

	Body position		Apparatus		Distraction			
	FM	MN	Lying	Seated	MNseat	MNly	FMseat	FMly
$\dot{V}O_2$	-8±17	-10±18	-4±11	-6±21	-3±25	-3±16	-16±20$^{¥}$	-6±13
$\dot{V}CO_2$	-4±19	-2±22	-3±13	-1±15	-4±18	-9±16	-14±22$^{¥}$	-7±16
RQ	0.02±0.07	0.04±0.07^{+}	0.00±0.08	0.02±0.04*	0.00±0.07	-0.03±0.07	0.00±0.06	-0.01±0.06
RMR	-50±123	-64±134	-28±67	-42±142	-26±168	-31±110	-110±152$^{¥}$	-49±98

Body position=lying down X seated; Apparatus=Face mask X Mouthpiece/nose-clip; Distraction=watching X no watching TV
¥ Statistically significant difference ($p<0.05$) between facemask seated watching TV and facemask seated not watching TV

* Statistically significant difference ($p<0.05$) between facemask and mouthpiece seated

\+ Statistically significant difference ($p<0.05$) between lying down and seated using mouthpiece

Mean $\dot{V}O_2$, $\dot{V}CO_2$, RQ, RMR, HR and RT3 counts are presented in Table 2.4. Repeated measures ANOVA indicated statistically significant differences for $\dot{V}O_2$ (p=0.01) and RMR (p=0.02), with TVMNSEAT showing higher values than TVFMLY, as shown in Table 2.4. There was no significant difference for $\dot{V}CO_2$, RQ, HR and RT3 counts in any combined situation.

Bland-Altman analysis showed a bias for $\dot{V}O_2$, $\dot{V}CO_2$, RQ and RMR between TVFMLY and TVMNSEAT, respectively, of -17.8±14.5 ml.min^{-1}, -8.8±14.5 ml.min^{-1}, 0.03±0.05 and -115.2±101.9 kcal.d^{-1}. Paired T-test indicated no significant differences between-subjects in mean RMR and mean RQ between morning and afternoon measurements.

Table 2.4 – Mean RMR, $\dot{V}O_2$, $\dot{V}CO_2$, RQ, HR and RT3 counts (± SD)

Situations	$\dot{V}O_2$ (ml.min^{-1})	$\dot{V}CO_2$ (ml.min^{-1})	RQ	RMR (kcal.d^{-1})	HR (bpm)	RT3 (counts)
TVMNLY	164±16	131±13	0.80±0.03	1144±107	77±9	13±18
TVFMLY	157±20	129±18	0.82±0.04	1098±142	76±9	11±19
TVMNSEAT	175±17§	138±13	0.79±0.04	1213±114$^{¥}$	81±14	64±96
TVFMSEAT	156±23	127±18	0.81±0.04	1087±161	80±13	53±102
MNLY	168±21	140±22	0.83±0.06	1175±152	78±11	51±133
FMLY	164±23	136±18	0.83±0.05	1147±157	77±9	18±27
FMSEAT	172±23	141±21	0.82±0.04	1197±161	84±12	54±67
MNSEAT	178±25	142±23	0.80±0.05	1239±176	85±13	107±147

§ Statistically significant difference (p=0.01) from TVFMLY

¥ Statistically significant difference (p=0.02) from TVFMLY

2.2.4 Discussion

RMR measurements have been conducted predominantly to determine dietary requirements and to inform dietary or physical activity prescriptions. Further, RMR measurements have been used to calculate $\dot{V}O_{2net}$ ($\dot{V}O_2$ exercise minus $\dot{V}O_2$ rest) with applicability in a large range of studies (Gaesser & Brooks, 1975; Frost, Bar-Or, Dowling, & Dyson, 2002; Ayub & Bar-Or, 2003; McCann & Adams, 2003; Sarton-Miller, Holman, & Spielvogel, 2003; Ekelund et al., 2004a). In a study of adults in our laboratory we have shown that different indirect calorimetry systems such as ventilated hood and mouthpiece and nose-clip can result in significant differences in RMR and $\dot{V}O_{2net}$ measures (Roffey et al., 2006). Considering the large variety of methodologies used across laboratories, and differences in the cited energy expenditure at rest in children (see Table 2.5), the present study compared RMR measurement in different body positions (sitting or lying), using different apparatus (mouthpiece and nose clip or facemask), and in different situations (TV viewing or no TV viewing). This is the first study to show the combined effect of body position, apparatus and distraction on children's RMR. RMR in this study did not appear to be significantly influenced by body position nor apparatus when each variable was considered independently. Analyses of distraction in three of four combinations indicated no difference between watching and not watching TV. However, subjects reported a preference for using the facemask and for watching TV.

One important finding of the present study was that when analysing each methodological parameter separately there was a statistically significant difference for the respiratory quotient (RQ) considering body position and apparatus, but no statistically significant difference for $\dot{V}O_2$, $\dot{V}CO_2$ and RMR (Table 2.3). The RQ under typical metabolic conditions with stable respiratory function is approximately 0.7 to 1. If the RQ is <0.7 or >1, prolonged starvation or excessive recent energy consumption should be suspected, with both events representing RMR protocols violation (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). Thermic effect of food (TEF) on afternoon measurements cannot be ruled out and should be considered as a study limitation. However we used RQ as a tool to detect some inaccurate measurements or RMR protocol violations. In our data collection the range of RQ for afternoon measurements was between 0.78 and 0.84 and no significant differences between-subjects in mean RQ and mean RMR between morning and afternoon measurements was verified. In future research may be advisable to control for meal composition before data collection when measurements were not performed in overnight fasting state.

Distraction comparisons were undertaken considering both body position and apparatus. Therefore, we considered four situations: a) TV or no TV seated + mouthpiece and nose-clip; b) TV or no TV lying down + mouthpiece and nose-clip; c) TV or no TV seated + facemask; and d) TV or no TV lying down + facemask. Interestingly, mean $\dot{V}O_2$, $\dot{V}CO_2$ and RMR measured not watching TV, seated and using a facemask were significantly increased compared to the same body position and apparatus watching TV, differences of 10.2%, 11.0% and 10.1%, respectively (Table 2.3). No significant difference was found in any other three distraction situations. An earlier study of distraction during RMR measurement (Klesges, Shelton, & Klesges, 1993) showed that TV viewing decreased RMR in obese and normal-weight children. However, the findings of another study (Dietz, Bandini, Morelli, Peers, & Ching, 1994) reported no difference between the TV viewing condition and no TV. More recently, research found no significant differences in

RMR between story listening or TV viewing and rest conditions (Cooper, Klesges, Debon, Klesges, & Shelton, 2006). Our results, in three of four combinations of environment, are in agreement with the more robust findings in the literature that TV viewing does not suppress RMR in children. However we are unaware of any study in the literature that has compared simultaneously body position, apparatus and distraction in children. Goran and Nagy (1996) provided another possible interpretation of the impact of TV viewing during RMR measurements in children. They suggested that independent of reductions in metabolic rate that perhaps children fidget less watching TV than when asked to sit quietly. We used movement and heart rate monitoring during all measurement sessions to monitor the resting state and to verify different levels of fidgeting among sessions. We found no significant differences for both indicators across all comparisons. These findings are in agreement with Dietz et al. (1994) who showed that increased fidgeting during RMR measurement was positively correlated with the minute-to-minute variation in metabolic rate data, but not to the absolute magnitude of metabolic rate. Evidence of an association between TV viewing and childhood obesity has recently been reported (Hancox & Poulton, 2006) however others (Cooper et al., 2006) have suggested that the most plausible explanation for potential weight change associated with TV viewing may not be associated with resting energy expenditure but with some combination of changes in energy intake and physical activity.

An early study (Hirsch & Bishop, 1982) showed that the choice of a mouthpiece or facemask differentially altered breathing patter. Breathing apparatus effect is not a simple consequence of a shift from oronasal to oral breathing, since the nose-clip under the mask did not change breathing pattern from that of mask alone. Comparisons between mouthpiece/nose-clip and facemask in the present study showed no significant differences in RMR measurements. Subjective ratings of breathing apparatus comfort indicated a preference for the face mask for all children studied. These findings are in agreement with metabolic studies in adults (Abadie & Carrol, 1993). In contrast, others have found that the use of a mouthpiece/nose-clip was preferable to the use of a facemask (Bukkens & McNeill, 1990). A study investigating the influence of mouthpiece/nose-clip practice procedure on RMR variability showed that variability may be minimized by practicing the procedure of acclimatising to the apparatus for 5 minutes immediately before the rest period

(Scott, 1993). In the present study we completed one period of adaptation because during the 20 min stabilization period children were all fitted with the apparatus used in the subsequent measurement. It is also important to consider the many differences in the facemasks used in previous studies. Design improvements in modern facemasks include characteristics of the materials used and the choice provided in sizes for better face adjustments, as well the use of silicone seal gel to improve the adherence and leakage prevention.

Numerous studies of RMR in non-obese children of similar ages to the present study have been completed with subjects measured lying and the RMR values have ranged between 1012±105 to 1420±157 kcal.d^{-1} (Maffeis, Zoccante, & Pinelli, 1990; Maffeis, Schutz, Micciolo, Zoccante, & Pinelli, 1993; Spadano, Must, Bandini, Dallal, & Dietz, 2003; Muller et al., 2004; Spadano, Bandini, Must, Dallal, & Dietz, 2004). In the seated position the RMR values ranged between 1133±241 to 1358±280 kcal.d^{-1} (Klesges et al., 1993; Dietz et al., 1994; Stensel, Lin, & Nevill, 2001; Nieman et al., 2005). The wide variability among different studies and therefore the lack of comparability using different methodological approach for RMR measurement prompted the current study. To the best of our knowledge no earlier study has compared the influence of body position on children's RMR. Weissman et al (1982) demonstrated differences in breathing patterns as a function of body position, with an increased ventilatory response in the sitting position. However, no statistically significant difference in $\dot{V}O_2$, $\dot{V}CO_2$ or RQ between the two postures was verified. This study showed no significant difference in values for RMR, $\dot{V}O_2$, $\dot{V}CO_2$ or RQ between the two body positions.

The most important finding of the current study was that the bias between TVMNSEAT and TVFMLY could indicate that when methodological issues are not stringently controlled, comparisons among RMR studies in children are not feasible. This could partially explain the reported differences in studies from different laboratories.

Reproducibility studies have showed intra-individual CVs between 2.6% and 5.8% in prepuberal children (Figueroa-Colon, Franklin, Goran, Lee, & Weinsier, 1996;

Goran & Nagy, 1996; Ventham & Reilly, 1999), 4.3% in adolescents (Rieper, Karst, Noack, & Johnsen, 1993) and between 2.8% and 7.3% in adults (Adriaens, Schoffelen, & Westerterp, 2003; Haugen, Melanson, Tran, Kearney, & Hill, 2003; McClave et al., 2003; Bader, Bosy-Westphal, Dilba, & Muller, 2005; Roffey et al., 2006). With regards to inter-individual CV the value of 9.1% was verified in children (Figueroa-Colon et al., 1996) and adolescents (Rieper et al., 1993). We showed intra- and inter-individual CV among every combined situation (Table 2.1). Our results are higher than reported intra- and inter-individual CVs in the literature mainly because for each measurement we introduced a new confounding variable, ie body positions, apparatus or distraction. Despite the necessary caution in these CV interpretations we believe that reported differences could be useful to support the need for a more stringent standardized protocol considering different combined situations for RMR measurements in children.

In conclusion, this study verified there were no differences in RMR measurements due to body position (seated or lying down) and apparatus (facemask and mouthpiece/nose-clip) when each variable was isolated. Analyses of distraction (TV or no TV) in three of four combinations among body position and apparatus indicated no difference between TV viewing or rest alone. However, significant differences were verified when analysing distraction combined with seated body position and using facemask. It is necessary to be cautious when comparing RMR measurements in children as studies have used different combinations of methodological issues addressed. Different combinations can result in increased bias and variability thereby reported differences among children's RMR measurement. When measuring RMR in children we hypothesised that maintenance of a resting state is likely to be enhanced by using TV watching to reduce boredom. Our study participants indicated a preference for the face mask as well as watching TV during RMR measures despite no significant effect of either protocol (apparatus or distraction) on values. However, to reduce the burden in research or in clinical measurements involving children, the combined objective and subjective data from this study provides evidence that the utilisation of facemask and watching TV should be considered as an effective approach to be used when designing RMR measurement protocols.

Table 2.5 - RMR – Non-obese children

Reference	***n***	**Age**	**Gender**	**Body position***	**RMR Kcal.d^{-1}**
Rodriguez et al. (2002)	59	7.8-16.6	M/F	Lying	1391±246
Dietz et al. (1994)	18	10.4±1.1	F	Seated	1160±193
	18	10.4±1.1	F	Seated TV	1133±241
Firouzbakhsh et al., (1993)	10	8-10	F		1092±139
	10	8-10	M		1158±168
	17	11-13	F		1398±169
	11	11-13	M		1452±195
Spadano et al. (2004)	10	9.9±1	F	Lying	1087±114
	26	9.9±1	F	Lying	1203±114
Hsu et al. (2003)	15	9.3±1.7	M/F	Lying	1223±184
Spadano et al. (2003)	17	8-12	F	Lying	1420±157
Maffeis et al., (1990)	14	8.6±1.1	M/F		1062±114
Maffeis et al. (1993)	48	7.6±1.5	M	Lying	1083±106
	49	7.8±1.2	F	Lying	1012±105
Maffeis et al. (1993)	10	8.6±0.4	M/F	Lying	1080±78
Roenmich et al. (2000)	14	10.9±1.0	M		1245±41
	13	10.2±1.4	F		1217±30
Spadano et al., (2005)	28	8-12	F		1249
Goran & Nagy (1996)	19	6.6±1.2	M/F	Lying TV	1050±151
Wurmser et al. (1998)	28	10.2±1.1	F	Lying-M	1203±155
	27	10.0±1.2	F	Lying-M	1070±138
Molnar & Schutz (1997)	116	13.1±1.7	M	Lying TV	1338±232
	119	13.1±1.7	F	Lying TV	1222±151
Treuth et al. (2000)	30	8.5±0.4	F	Lying	1070±117
	44	8.5±0.4	F	Lying	1087±100
	27	8.5±0.4	F	Lying	1125±109
Kirkby et al. (2004)	170	5.9±0.2	M	Lying TV	1129±147
	137	5.9±0.2	F	Lying TV	1068±127
Klesges et al. (1993)	16	10.2±1.1	F	Seated TV	1254±178
	16	10.2±1.1	F	Seated	1358±280

Present Study	14	10.1±1.4	F	Lying $_{MN}$TV	1144±107
	14	10.1±1.4	F	Lying$_{FM}$ TV	1098±142
	14	10.1±1.4	F	Seated$_{MN}$ TV	1213±114
	14	10.1±1.4	F	Seated$_{FM}$ TV	1087±161
	14	10.1±1.4	F	Lying$_{MN}$	1175±152
	14	10.1±1.4	F	Lying$_{FM}$	1147±157
	14	10.1±1.4	F	Seated$_{MN}$	1197±161
	14	10.1±1.4	F	Seated$_{FM}$	1239±176

* Blank = not cited
Laying-M = listening music
Laying TV or seated TV = watching TV
MN = Mouthpiece & Nose clip
FM = Facemask

Chapter III –DETERMINANTS OF WALKING ENERGY EXPENDITURE IN CHILDREN

3.1 Literature Review

Several investigators have demonstrated that the energy expended during weight-bearing physical activity increases with higher body weight. Levine et al. (2002) established that although the energy expenditure of walking tasks was proportional to body weight, activities of sitting and standing were not (Levine, Schleusner, & Jensen, 2000). Schoeller and Jefford (2002) also showed that the energy cost of some typical light activities which are largely arm and leg movements, also increase in proportion to body weight, although not all involve movement of the whole body. However, Levine (2004) in a review paper showed that it is less clear whether work efficiency varies with body composition, independent of body weight.

Levine (2004) speculated that ambulatory energy expenditure increases and accounts for the vast majority of the changes in non-exercise activity thermogenesis. He suggested that the mechanism that drives ambulatory energy expenditure is pivotal to understanding non-exercise activity thermogenesis. The energy cost of selected movements for overweight children is greater than for normal-weight children (McGraw, McClenaghan, Williams, Dickerson, & Ward, 2000), and further, the overweight state rather than any metabolic defect may explain the increased energy cost of walking and running in the obese (Katch, Becque, Marks, Moorehead, & Rocchini, 1988). An increase in physical activity energy expenditure (AEE) and PAL is more likely due to an increase in body size or body weight, and therefore these estimates may not be the best indicators of the total amount of physical activity in comparison to groups who differ in body size or in longitudinal assessments during growth (Ekelund, Yngve, Brage, Westerterp, & Sjostrom, 2004b).

Kleiber’s law is one of the most important and best-known laws in bioenergetics (Hall, 1999). Kleiber first surveyed resting energy expenditure (REE) estimates for mature mammals from rats to steers with a ~ 2,800-fold difference in body size. As a fundamental physical characteristic, body mass was applied early in the development of REE-body composition models (Wang, O'Connor, Heshka, & Heymsfield, 2001), and REE is usually the largest portion of total energy expenditure.

The conventional ratio methods used to express $\dot{V}O_2$ are in absolute terms (ml.min^{-1}) or in relative terms expressed linearly (ml.kg^{-1}.min^{-1}). As noted earlier by Katch and Katch (1974), a negative association between these variables is typically reported. Consequently, dividing $\dot{V}O_2$ by body weight does not create a mass independent variable. The influence of mass is not generally removed by the ratio method, thus penalising heavier individuals (Loftin, Sothern, Trosclair, O 'Hanlon, Miller, & Udall, 2001). Appropriate scaling for differences in body size is a fundamental requirement for the clarification of how exercise performance changes with normal growth and maturation (Welsman & Armstrong, 2000).

In the literature one can find different approaches for matching walking economy between adults and children, including by body surface area and body size (Ebbeling, Hamill, Freedson, & Rowland, 1992; Rogers, Olson, & Wilmore, 1995; Maliszewski & Freedson, 1996; Heil, 1997); by height and weight (Allor, Pivarnik, Sam, & Perkins, 2000); by energy-speed relationships (Waters, Lunsford, Perry, & Byrd, 1988); by allometric scaling methods (Armstrong, Kirby, McManus, & Welsman, 1997a; Heil, 1997; Walker, Murray, Jackson, Morrow, & Michaud, 1999; Armstrong & Welsman, 2001; Loftin et al., 2001) and by total body mass (Ayub & Bar-Or, 2003). Excluding the last authors, all studies used mathematical procedures for matching the participants and there was no consistency in results generated from these studies.

Ebbeling et al. (1992) reported that there were no significant differences between children and adults when $\dot{V}O_2$ was normalised by body surface area rather than body mass, but Maliszewski and Freedson (1996) demonstrated that running economy comparisons between boys and men are dependent upon the test model used. Murray et al. (1993) suggested that gender differences in peak $\dot{V}O_2$ in adolescents is due in part to gender differences in body fatness, but Walker et al. (1999) found no gender difference in sub-maximal and running energy cost in adolescents, even though males and females differed in skinfold thickness. However it has been illustrated from steady-state measures that such differences in the oxygen cost between adults and children are accentuated by the inappropriate normalisation procedure of the simple body mass ratio (Fawkner & Armstrong, 2003).

Allor et al. (2000) in a cross-sectional research design to examine differences in walking and running economy between adolescent and young adult females matched for height and body weight, verified that matching by body size alone does not remove differences in exercise economy during treadmill walking and running exercise.

The use of allometric models to derive truly size-independent exercise performance measures has been demonstrated to be theoretically and statistically superior to the simple ratio standard in analysing size-related function (Armstrong et al., 1997a). A number of studies (Welsman, Armstrong, Nevill, Winter, & Kirby, 1996; Armstrong & Welsman, 2001; Loftin et al., 2001) using allometric scaling have suggested that this method should be considered when comparing $\dot{V}O_2$ peak of obese youth with normal-weight youth. Previous research has found allometric scaling to reduce the effect of body size in youths and adults with exponents ranging from 0.37 to 1.10, with virtually no consistency between studies (Armstrong et al., 1997a; Heil, 1997; Rowland, 1997a; Welsman & Armstrong, 2000; Armstrong & Welsman, 2001). According to the theory of geometric similarity, as human power output is proportional to stature^2 or body mass$^{0.67}$, peak $\dot{V}O_2$ should also be proportional to mass$^{0.67}$ (Astrand, 1976). When investigating functional changes in peak $\dot{V}O_2$ the use of the theoretical exponent of 0.67 (ml.kg$^{-0.67}$.min^{-1}) to normalise data is recommended. It was noted that in heterogeneous groups, utilising stature as a continuous covariate is an important consideration, allowing any disproportionate increase in peak $\dot{V}O_2$ associated with body size to be identified (Welsman et al., 1996).

Nevill et al. (1992) suggested that for weight-bearing activities, the likely body size denominator will be total body mass. Some have showed that the exponents approached 1.0 as the level of energy expenditure increased, independently of the weight dependence of the activity (Zakeri, Puyau, Adolph, Vohra, & Butte, 2006).

Scaling to body mass implies that the components of mass have the same physiologic importance and that the composition of mass is similar between individuals. The impact of variations in body fat content might be expected to affect scaling factors for endurance performance in a population of subjects heterogeneous for body composition (Rowland, 1996). Vanderburgh and Katch (1996) indicated that increased levels of body fat may lead to a reduction in the mass exponent because a larger portion of the body mass is adipose tissue. Despite differences between the diverse exponents suggested, the allometric modelling does not always reduce the bias of body size.

Interestingly, Prentice et al. (1996) suggested that it is impossible to develop a generally applicable adjustment factor since the appropriate exponent for body weight is itself dependent on each individual’s daily mix of activities with the exponent rising as people are more active and undertake more weight-dependent activities.

A possible explanation for the conflicting results in walking energy expenditure among people with large variation in body size may be due to the statistical adjustment methods which may not adequately control for body composition.

During growth and maturation $\dot{V}O_{2peak}$ is highly correlated with body size, and the independent effects of chronological age, maturation and sex on $\dot{V}O_{2peak}$ must be examined to minimise the confounding influence of body size (Armstrong & Welsman, 2001). Marinov et al. (2002) showed that the absolute metabolic cost of exercise was higher in an obese group compared with control subjects, and obese children had an increased awareness of fatigue that may further limit their physical capacity. Fatness and excess body weight do not necessarily imply a reduced ability to consume oxygen, but excess fatness does have a detrimental effect on sub-maximal aerobic capacity (Goran, Fields, Hunter, Herd, & Weinsier, 2000).

Hoos et al. (2003a) showed in a meta-analysis that AEE and PAL values in children aged 3-16 years increase with age in both sexes during growth, but whether there is an increase in total amount of physical activity per se is doubtful. Not only can the intensity of the same activity be extremely different between individuals, but different absolute levels of aerobic fitness between individuals can have important implications for the translation from certain activities into energy expenditure (Twisk, 2001).

The factors linked to higher sub-maximal energy expenditure in children during walking include: greater REE (Rowland, 1996); greater stride frequency (Unnithan & Eston, 1990); differences in discrete kinematic variables (Ebbeling et al., 1992) and differences in more “global” measures of work done, such as total body mechanical work and power (Frost, Dowling, Bar-Or, & Dyson, 1997; Unnithan, Dowling, Frost, & Bar-Or, 1999), however none of these factors provides a complete explanation for the higher oxygen consumption per kilogram of body mass in younger children than older children or adults when walking or running at the same speed (Frost et al., 2002).

It is necessary to be cautious regarding the identification of an ‘ideal’ scaling factor(s) for physiologic variables as such factors may only provide a tool for intra- and inter-subject comparisons, without implications for causality (Rowland, 1996). Therefore, it should be emphasised from the outset that there is no universally “correct” method of scaling, neither is any one of these methods necessarily “incorrect” in all instances. All of the methods are constrained by underlying statistical assumptions that, if ignored, may confuse interpretations based upon them (Welsman & Armstrong, 2000).

The mechanically optimal walking speed is the speed at which the transfer between gravitational potential energy and kinetic energy is maximal, and the weight specific work necessary to maintain the movement of the center of mass for a given distance is minimal (Cavagna, Franzetti, & Fuchimoto, 1983). *Economy* is defined as the amount of energy necessary to perform a given submaximal task. The less energy required to perform the task, the greater the economy. Given the differences in running mechanics and relative work intensities when children run at the same speed as adults, this model has limited application for identifying developmentally-based physiological differences (Maliszewski & Freedson, 1996). The use of walking speeds corresponding to percentages of average walking speed during the One-mile Walk Test to examine efficiency during walking in children and adults has been reported (Ebbeling et al., 1992), in an attempt to ensure that the two groups would be walking at similar relative intensities.

One of the key steps in indirect calorimetry is the determination of the composition of the metabolic mixture of carbohydrate and fat oxidised using a non-protein respiratory quotient. Tables based on the RQ indicate that for a given ratio rate of CO_2 produced / rate of O_2 utilized, the percentage of energy provided from carbohydrate versus fat oxidation, and the energy equivalent of oxygen (Peronnet & Massicotte, 1991).

The pattern of substrate utilisation of an individual at any point in time depends on the crossover between the exercise intensity-induced responses, which increase carbohydrate utilisation, and the endurance training-induced responses, which promote lipid oxidation. The crossover point is identified as the power output which energy derived from oxidation of carbohydrate-based fuels predominates over that derived from lipids, with further increases in power eliciting a relative increment in energy from carbohydrate and a relative decrement in energy from lipid oxidation (Brooks & Mercier, 1994).

Many factors including intensity, duration and type (aerobic vs. anaerobic) of exercise, energy expended during exercise, and individual fitness level impact the amounts of fat oxidised at any given time (Hansen, Shriver, & Schoeller, 2005). The exact relationship between maximal fat oxidation rate and $\dot{V}O_{2max}$ is not known but it can be explained by the fact that when exercising at the same relative intensity, a higher $\dot{V}O_{2max}$ corresponds to a higher absolute work rate resulting in higher energy expenditure and, if RER is equal, a higher amount of fat being oxidised (Stisen, Stougaard, Langfort, Helge, Sahlin, & Madsen, 2006).

Studies of adults have shown a distinct relationship between intensity of common activities, such as walking, running or biking, and fat oxidation (Achten, Gleeson, &

Jeukendrup, 2002; van Aggel-Leijssen, Saris, Wagenmakers, Senden, & van Baak, 2002). To optimise fat oxidation during walking, it could be helpful to regulate the oxidation during walking to obtain maximal fat oxidation (Maffeis et al., 2005). This group has shown that moderately intense exercise promotes higher fat oxidation rates, expressed as a percentage of total energy expenditure, than more strenuous exercise in boys. Brandou et al. (2006) also showed that puberty alters the balance of substrate oxidation during exercise in markedly obese children, with postpuberal children relying less on fat oxidation than prepuberal individuals.

RER differences between girls and women (Martinez & Haymes, 1992) and boys and men (Maliszewski and Freedson, 1996) at the same relative work intensity have been reported, suggesting a higher reliance on fats and lower carbohydrate use in children, supporting the hypothesis that children tend to have lower glycolytic capacity than adults.

3.2 Treadmill adaptation and verification of self-selected walking speed: A protocol for children.

Modified from: Amorim PRS, Byrne NM, Hills AP (paper under review) ‘Treadmill adaptation and verification of self-selected walking speed: A protocol for children.’

3.2.1 Introduction

Indirect calorimetry is often used to measure energy expenditure during treadmill walking. However, assessment of individual and group differences in metabolic energy expenditure using oxygen uptake requires that individuals are comfortable with, and can accommodate to, the equipment being utilised. To ensure the data from a treadmill walking protocol does not simply reflect an adaptation to the test, it has traditionally been assumed that participants should be habituated to walking on the treadmill before testing (Maltais, Bar-Or, Pierrynowski, & Galea, 2003). Participants with insufficient exposure to the apparatus may be unable to make the necessary adjustments to achieve a stable and consistent gait pattern and therefore introduce bias to the measurements (Frost, Bar-Or, Dowling, & White, 1995). The literature is not consistent about the length of this exposure with wide variability in exposure time reported, 15-20 sec (Frost et al., 1995), 2 min (Schieb, 1986), 2-3 min (Frost et al., 1997), 5 min (Morgan, Tseh, Caputo, Craig, & Keefer, 1997; Tseh, Caputo, Craig, Keefer, Martin, & Morgan, 2000; Keefer, Tseh, Caputo, Apperson, McGreal, & Morgan, 2005) and 8 min (Wall & Charteris, 1980). Despite the reported variability in time, Morgan et al. (1997) showed that the majority of gait adjustments for young children occurred during the initial 10 min walking bout or the familiarisation sessions.

Controversy exists regarding whether walking tests should be performed on a treadmill or over-ground. While some studies have found significant differences in energy cost (Pearce, Cunningham, Donner, Rechnitzer, Fullerton, & Howard, 1983; Swerts, Mostert, & Wouters, 1990) and performance (Stevens, Elpern, Sharma, Szidon, Ankin, & Kesten, 1999) using these different modalities, others have reported equivalence in measures (Peeters & Mets, 1996). Advantages have been cited for each mode of testing. For example, treadmill testing allows easy concurrent measurement of physiologic data, such as gas exchange, whereas over-ground walking is easier to implement, requires minimal equipment, more closely replicates everyday walking and is clinically applicable. However, many of the reported comparisons between modalities were performed with the elderly or in a rehabilitation context with patients who had cardiac or pulmonary disease, most with limited activity levels and exercise capacity (Solway, Brooks, Lacasse, & Thomas, 2001).

The terms "self-selected, individual or comfortable walking pace or speed" are commonly used in the literature (Frost et al., 1997; Jeng, Liao, Lai, & Hou, 1997; Wergel-Kolmert & Wohlfart, 1999; Maltais et al., 2003; Browning & Kram, 2005; Browning, Baker, Herron, & Kram, 2006; Hills, Byrne, Wearing, & Armstrong, 2006). The self-selected speed (SS) should be determined using a consistent protocol to ensure that the representative walking speed of an individual is identifiable and subsequently applied in treadmill walking projects. However, we have been unable to find a detailed description of a protocol to determine SS using the treadmill. Further, we have not found data to verify the "true" achievement of this individual speed of reference.

We hypothesised that SS determined using an indoor over-ground protocol would be reproducible on the treadmill. Further, using a 10 min familiarisation protocol based on SS one can verify the achievement of preferred walking speed and therefore assist in the improvement of the reliability of the oxygen uptake measures between tests.

2.2.2 Materials and methods

3.2.2.1 Participants

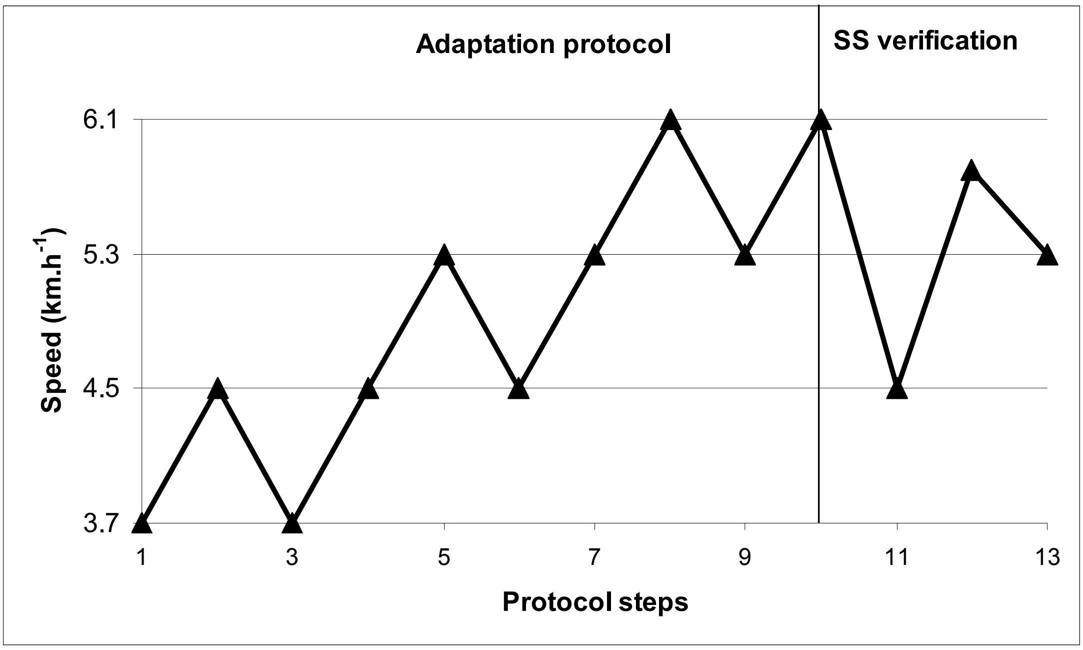
Experiments were carried out on 27 children aged 8-12 (mean age = 10.3±1.2 yr, mean mass = 38.6±11.4 kg, mean height = 145.0±10.0 cm) recruited from the Brisbane metropolitan area through advertisements distributed in schools. Parents or caregivers of respondents were interviewed by telephone and children were classified as eligible based on age and medical history. Exclusion criteria included individuals with known medical conditions such as type I or type II diabetes mellitus or any metabolic disease. Eligibility for the study was dependent on participants being ambulatory and their ability to tolerate any necessary instrument or apparatus used during testing sessions. All participants were in good health and were not taking medications known to influence metabolism. The study protocol was approved by the Human Research Ethics Committee at the Queensland University of Technology. Participants and their parents or caregivers gave written informed consent prior to their inclusion in the study.

3.2.2.2 Study Design

The SS was determined on a level surface by measuring the time required to walk 20m. Trials 1 and 7 of 7 trials were disregarded and after discounting the first and last 5 m of trials 2-6, SS was calculated. After determination of SS each participant had a 10 min treadmill familiarisation period, using participants' SS, 2 slower and one faster pace (1.6 and 0.8 km.h^{-1} less than SS and 0.8 km.h^{-1} faster than SS). This approach was used to verify the achievement of the SS using the following protocol:

Start with the slowest speed (1 min) then increase by 0.8 km.h^{-1} (1 min), reduce to the slowest speed (30 sec) and then increase again to the second slowest speed (30 sec). Ask the participant which of these 2 speeds was the most comfortable. Increase to SS (1 min), reduce again to the second slowest speed (30 sec) then increase to SS (30 sec). Again ask participant to rate which of the speeds was the most comfortable. Increase to the faster speed (1 min), reduce again to SS (30 sec) then increase again to the faster speed (30 sec). Ask the participant which of the third set of speeds was more comfortable. After these three initial speed comparisons, reduce the speed to the first chosen slower speed (1 min), increase to the faster chosen speed (1 min) and finally reduce to the second chosen speed (1 min). Again ask the participant which was the most comfortable treadmill walking speed. If the over-ground selected speed was not matched with the comfortable treadmill pace, the final SS was set as the comfortable treadmill pace. The adaptation protocol and SS verification is graphically represented in Figure 3.1.

Figure 3.1 – Adaptation protocol and SS verification scheme



A sub-sample of 14 participants completed 3 test sessions over 2 days. One day included both morning (T1) and afternoon (T2) sessions and the second day included an afternoon (T3) session only, one week apart.

3.2.2.3 Data Collection

The treadmill sub-maximal protocol included the same speeds as in the familiarisation protocol. Each stage was 6 min in duration followed by 5 min rest between each velocity. Participants were fitted with a nose and mouth paediatric face mask (FM) (series model 8950 - Hans Rudolph, Inc.) with a two-way breathing T-shaped valve and pneumotach. Ventilation (V_E), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were assessed from breath-by-breath samples using a MOXUS Modular $\dot{V}O_2$ System. A digital pedometer was attached to the left hip (DigiWalker SW-700) during the treadmill session. Over-ground step length was directly measured by counting the number of gait cycles during the SS determination.

3.2.2.4 Data Analyses

To represent data from SS measured over-ground and on the treadmill, plus for within- and between-day $\dot{V}O_2$ (ml.kg^{-1}.min^{-1}), a plot was made of the differences between the measurements against their mean using the Bland and Altman method (Bland & Altman, 1986). Paired-T test was used between over-ground and treadmill SS. To analyse within-day and between-day variability, coefficient of variation (CV) were determined. Individual CV values were averaged to obtain a mean group CV value for $\dot{V}O_2$ and steps. Repeated measures ANOVA was used for verify CV as well as step length differences followed by a *Tukey Post Hoc* test. A significance level of $p<0.05$ was set for all analyses.

3.2.3 Results

As the main focus of this paper is on SS, only data from this speed are reported. Mean SS measured over-ground was 5.26±0.5 km.h^{-1}, while on the treadmill mean SS was 5.20±0.5 km.h^{-1}. Mean difference was 0.06 (95% CI: -0.03 to 0.14, SEM: 0.04). Paired T-test showed correlation of 0.90 ($p<0.001$) but no significant differences between SS determined over-ground and on a treadmill.

Mean $\dot{V}O_2$ at SS was 16.0±4.0, 15.5±4.1 and 16.4±3.6 ml.kg^{-1}.min^{-1} for test 1, 2 and 3 respectively. Mean within-day $\dot{V}O_2$ difference was 0.32 ml.kg^{-1}.min^{-1} (95% CI: -0.40 to 1.04, SEM: 0.33, CV: 3.8%). Mean between-day $\dot{V}O_2$ difference was -0.57 ml.kg^{-1}.min^{-1} (95%CI: -1.36 to 0.22, SEM 0.36, CV: 4.4%).

Mean treadmill step length was 0.64±0.06 m, 0.65±0.06 m, 0.65±0.09 m, respectively for test 1, 2 and 3. Within- and between-day treadmill step-length CV was consistent at 2.0%. In contrast, over-ground step length was 0.68±0.07 m. Repeated measures ANOVA showed no significant differences in step lengths among treadmill tests, but significant difference ($p<0.05$) was found between over-ground and treadmill step length for all three tests.

Repeated measures ANOVA showed no significant CV differences for $\dot{V}O_2$ and steps.

3.2.4 Discussion

Commonly, over-ground protocols have employed gait tracks of 7-10 m in length and reported good between-day reliability using the preferred walking speed (Wearing, Urry, Smeathers, & Battistutta, 1999; Batey, Rome, Finn, & Hanchard, 2003). However, few studies have applied an over-ground walking test to define preferred treadmill walking speeds for children and these studies have used an outdoor running track of 400 m (Frost et al., 1995) or a 5 m distance in the middle of a walkway (Jeng et al., 1997). Data from over-ground SS should be more representative of the real walking "pace" used in daily life. No study has verified the reliability of the SS determined over-ground and subsequently used that speed to establish a treadmill familiarisation protocol. This may not be surprising because the SS is generally subjectively determined as reported by Maltais et al. (2003).

Our results showed very good correlations and no significant differences between SS determined over-ground and on a treadmill. The mean difference between over-ground and treadmill step length was 0.03 m. This value, despite being statistically significant, has no clinical importance as the mean of the within- and between-day $\dot{V}O_2$ differences were not significantly different from zero with 95% of differences less than two standard deviations, a criteria for acceptable reproducibility (Bland & Altman, 1986) as well as lower and no significant CV difference. The similar results for step length with no significant differences among treadmill tests and lower and no significant variability within- and between-day is indicative of the efficacy of our adaptation protocol.

Without strict planning, SS determination could be time consuming, increase the rating of perceived exertion, as well as potentially increase boredom in children. These results suggest that SS speed determined over-ground is reproducible on a treadmill and our 10 min familiarisation protocol based on this speed was able to provide enough exposure to achieve accommodation to the treadmill and, consequently, reliable within- and between-day $\dot{V}O_2$ in children performing treadmill exercise.

3.3 Within- and between-day repeatability and variability in children's physiological responses during submaximal treadmill exercise

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Modified from: Amorim PRS, Byrne NM, Hills AP (paper under review) 'Within- and between-day repeatability and variability in children's physiological responses during submaximal treadmill exercise.'

3.3.1 Introduction

Knowledge of the stability of walking energy expenditure in children is of particular importance to researchers interested in understanding differences in oxygen consumption ($\dot{V}O_2$) between groups of participants who vary in sex, age, disease status, activity level, and motor function. An appreciation of common walking characteristics is also important in the assessment of the efficacy of treatments designed to lower the energy cost of walking (Keefer et al., 2005) and specific physical activity prescriptions. One of the major aims of physical training is to restore or increase physical capacity in health and disease. Therefore, it is of particular importance to have reliable measures of physical work capacity to plan individual or group training (Wergel-Kolmert & Wohlfart, 1999).

Energy expenditure during walking influences the self-selected walking speed of normal weight individuals with the rate of metabolic energy consumption increasing curvilinearly with walking speed. Accordingly, the energy consumption per unit distance results in a U- or J-shaped curve when plotted versus walking speed (Zarrugh, Todd, & Ralston, 1974; Margaria, 1976). The gross cost of transport is of particular interest. At lower walking speeds this cost increases (DeJaeger, Willems, & Heglund, 2001) and faster walking speeds incur disproportionately greater metabolic rates (Browning & Kram, 2005). Self-selected walking speed is commonly identified as the most efficient walking speed; with increased efficiency defined by lower $\dot{V}O_2$ per unit mechanical work (Hoyt & Taylor, 1981; Taylor, Heglund, & Maloiy, 1982; Hreljac, 1993; Browning & Kram, 2005; Browning et al., 2006).

Controversy exists regarding whether walking tests should be performed on a treadmill or over-ground. While some studies have found significant differences in energy cost (Pearce et al., 1983; Swerts et al., 1990) and performance (Stevens et al., 1999) using these different modalities, others have reported equivalence in measures (Peeters & Mets, 1996). Advantages have been cited for each mode of testing. Treadmill testing allows easy concurrent measurement of physiologic data, such as gas exchange, whereas over-ground walking is easier to implement, requires minimal equipment and more closely replicates everyday walking. However, many of the earlier comparisons between these modalities were performed with the elderly or with patients who had cardiac or pulmonary disease and in rehabilitation, most with limited activity levels and exercise capacity (Solway et al., 2001).

Indirect calorimetry is often used to measure energy expenditure during walking on a treadmill. However, assessment of individual and group differences in metabolic energy expenditure using oxygen uptake requires that individuals are comfortable with, and can accommodate to, the equipment utilised. To ensure the data from a treadmill walking protocol does not simply reflect an adaptation to the test, it has traditionally been assumed that participants should be habituated to walking on the treadmill before testing (Maltais et al., 2003). Participants with insufficient exposure to the apparatus may be unable to make the necessary adjustments to achieve a stable and consistent gait pattern and therefore introduce bias to the measurements (Frost et al., 1995). It has been suggested that these adjustments occur in two phases: accommodation, or the development of an essentially normal and fairly stable gait pattern, may occur in the initial exposure. Habituation is not achieved until kinematic analysis of gait reveals no significant within-day or between-day differences from stride to stride (Charteris & Taves, 1978). Few studies have examined the within-and between-day stability of $\dot{V}O_2$ values during treadmill walking in able-bodied children (Frost et al., 1995; Tseh et al., 2000) and little is known about the amount of practice necessary and the optimal protocol required to achieve habituation.

Self-selected walking speed should be determined using a consistent protocol to ensure that the representative walking speed of an individual is identifiable and confidently applied in treadmill walking projects. Commonly, protocols have employed gait tracks of 7-10 m in length and reported good between-day reliability in the preferred walking speed (Wearing et al., 1999; Batey et al., 2003). Few studies have applied an over-ground walking test to define preferred treadmill walking speeds for children (Frost et al., 1995; Jeng et al., 1997) and subsequently used that speed to establish a familiarisation protocol. Such an approach enables one to verify the achievement of preferred walking speed and therefore assist in the improvement of the reliability of the oxygen uptake measures between tests. As walking is the most commonly reported physical activity and is a natural, accessible and safe form of movement, we investigated the within- and between-day variability in oxygen uptake during treadmill walking using the preferred speed and three derived velocities in children. We also determined the feasibility of a familiarisation protocol based on these velocities to achieve a reliable $\dot{V}O_2$ measurement.

3.3.2 Materials and Methods

3.3.2.1 Participants

Experiments were carried out on 14 children aged 8-12 (mean age = 10.1±1.4 yr, mean mass = 34.5±6.5 kg, mean height = 144±10.2 cm) recruited from the Brisbane metropolitan area through advertisements distributed in schools. Parents or caregivers of respondents were interviewed by telephone and children were classified as eligible based on age and medical history. Exclusion criteria included individuals with known medical conditions such as type I or type II diabetes mellitus or any metabolic disease. Eligibility for the study was dependent on participants being ambulatory and their ability to tolerate any necessary instrument or apparatus used during testing sessions. All participants were in good health and were not taking medications known to influence metabolism. The study protocol was approved by the Human Research Ethics Committee at the Queensland University of Technology. Participants and their parents or caregivers gave written informed consent prior to their inclusion in the study.

3.3.2.2 Study design

The self-selected speed (SS) determination and treadmill accommodation were performed using the same procedure described earlier in session 3.2.

Each participant completed 3 test sessions over 2 days. One day included both morning (T1) and afternoon (T2) sessions and the second day included an afternoon (T3) session only, one week apart. Morning sessions were conducted between 7:00 and 8:30 am, and afternoon sessions were scheduled between 3:30 and 5:00 pm to coincide with the time heart rate reaches its approximate peak in circadian rhythm during waking hours (Winget, DeRoshia, & Holley, 1985). Morning session participants were instructed to remain in an overnight fasted state and participants in afternoon sessions were instructed to finish their lunch at least 4 hours before the scheduled session time and avoid strenuous physical activity on the day of the test.

3.3.2.3 Data Collection

The treadmill protocol included 2 slower speeds (1.6 and 0.8 $km.h^{-1}$ less than SS), SS, and a faster speed (0.8 $km.h^{-1}$ faster than SS). Each stage was 6 min and followed by 5 min rest between each velocity.

Data collection took place in a thermo-regulated environment. Participants were fitted with a nose and mouth pediatric face mask (FM) (series model 8950 - Hans Rudolph, Inc.) with a two-way breathing T-shaped valve and pneumotach. To improve the seal between face and mask an Ultimate Seal^{TM} gel was used. Participants were also fitted with a Polar heart rate transmitter (Polar WearLink Transmitter, Polar Electro Oy, Finland) and a receiver (model S610 Polar Electro Oy, Finland). Ventilation (V_E), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were assessed from breath-by-breath samples using a MOXUS Modular $\dot{V}O_2$ System. A turbine attached to the inspired side of a Hans Rudolph two-way non-rebreathing valve measured volume and airflow, and it was verified before each measurement. Oxygen concentration of expiration was measured via an S-3A Oxygen Analyser sensor that contains a stabilised zirconia high-temperature electrochemical cell which has an inherently rapid response to changes in oxygen concentrations from 0-100%. Carbon dioxide concentration of expiration was measured via a CD-3A Carbon Dioxide Analyser which contains infrared optics with filters, a chopper and a cooled lead selenide detector with preamplifier capable of continuous and accurate analysis of carbon dioxide concentrations from 0-15%. A Model R-1 Flow Control that contains a flow meter, pump and needle valve was responsible for drawing the sample gases through the sensors and then venting them to the room. Oxygen and carbon dioxide gas analysers were calibrated before each measurement using known standard gas concentrations.

3.3.2.4 Data Analysis

A preliminary stabilisation period of 3 min was allowed for each speed followed by a 3 min sampling period. Mean $\dot{V}O_2$ data from minutes 5 and 6 of each bout were compared to assess whether the children were at steady state.

To analyse the within- and between–day repeatability of measurements, a plot was made of the differences between the measurements against their mean according to the method of Bland and Altman (Bland & Altman, 1986) for $\dot{V}O_2$ (ml.kg^{-1}.min^{-1}), GE [$\dot{V}O_2$ (ml.kg^{-1}.min^{-1}) divided by speed (m.min^{-1})] and HR (bpm). To analyse within-day and between-day variability, coefficients of variation (CV) were determined for each child across exercise bouts. Individual CV values were averaged to obtain a mean group CV value for $\dot{V}O_2$, GE and HR per speed. Repeated measures ANOVA followed by a *Tukey Post Hoc* test at a significance level of $p<0.05$ was used to verify differences in CV.

3.3.3 Results

The range of preferred speeds used to derive all other velocities on the treadmill was between 1.25 and 1.56 m.sec^{-1} (mean self-selected speed = 1.47±0.15 m.sec^{-1}). Of major importance in this study was the measurement of $\dot{V}O_2$ with participants in a steady state. Using the criterion for steady state established by Frost et al. (1995) no participants differed by more than 2 ml.kg^{-1}.min^{-1} in $\dot{V}O_2$ between the last two minutes in any 6 min exercise bout. The means and standard deviations for $\dot{V}O_2$ and heart rate per speed and test are displayed in Table 3.1. Average GE per speed is shown in Figure 3.2.

Table 3.1- Mean and standard deviation for $\dot{V}O_2$ *and HR per speed by test*

Test	**< 1.6 km.h^{-1}**		**< 0.8 km.h^{-1}**		**SS**		**> 0.8 km.h^{-1}**	
	$\dot{V}O_2$	HR	$\dot{V}O_2$	HR	$\dot{V}O_2$	HR	$\dot{V}O_2$	HR
T1	12.0±2.4	106±13	13.3±3.2	112±15	16.0±4.0	121±16	19.0±5.0	135±19
T2	12.3±2.9	107±12	13.6±3.2	113±13	15.5±4.1	122±15	18.6±4.4	137±18
T3	12.5±2.6	108±16	14.0±3.0	114±16	16.4±3.6	122±14	19.5±4.7	137±19

$\dot{V}O_2$ *(ml.kg^{-1}.min-^{1})= oxygen uptake; HR (bpm) = Heart rate SS = self-selected speed*

Figure 3.2- Average (±SEM) gross economy per speed

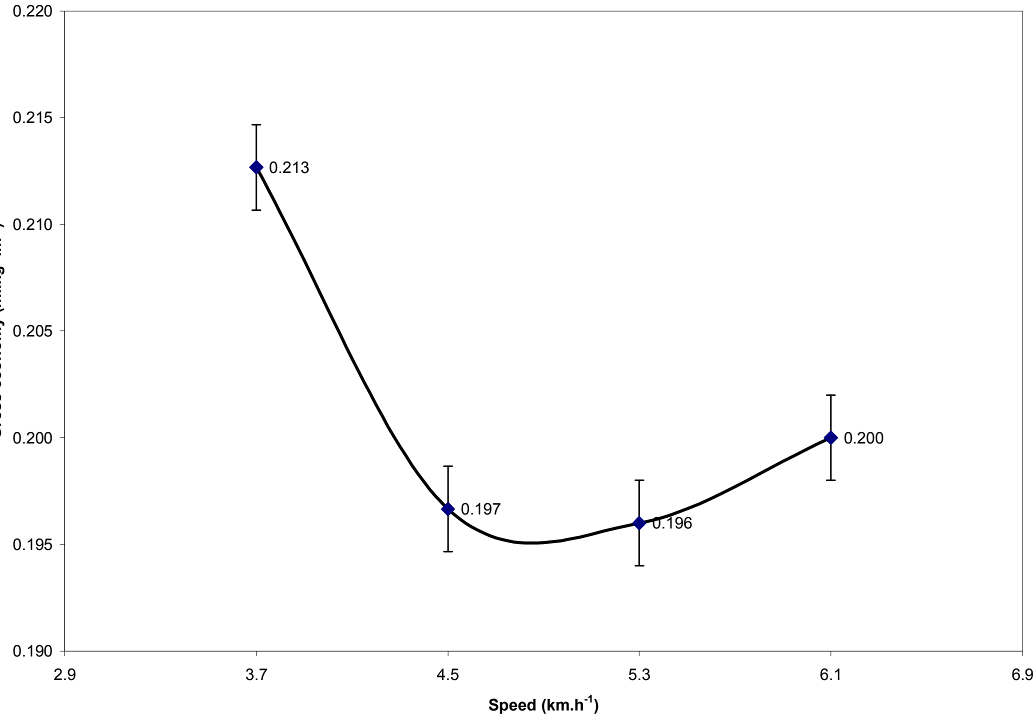


Figure 3.3 illustrates within-day plots of the difference for $\dot{V}O_2$ at SS and Figure 3.4 for between-day. The mean of the within-day differences was not significantly different from zero at any speed for $\dot{V}O_2$, GE and HR. An analogous analysis was made for between-day data and no significant difference was verified for any studied variable. Within-and between-day repeatability was quantified as two standard deviations. Our data from $\dot{V}O_2$, GE and HR at all speeds showed that 95% of

differences were less than two standard deviations. Data from these analyses are shown in Table 3.2.

Figure 3.3 – Within-day difference in $\dot{V}O_2$ (ml.kg^{-1}.min^{-1})

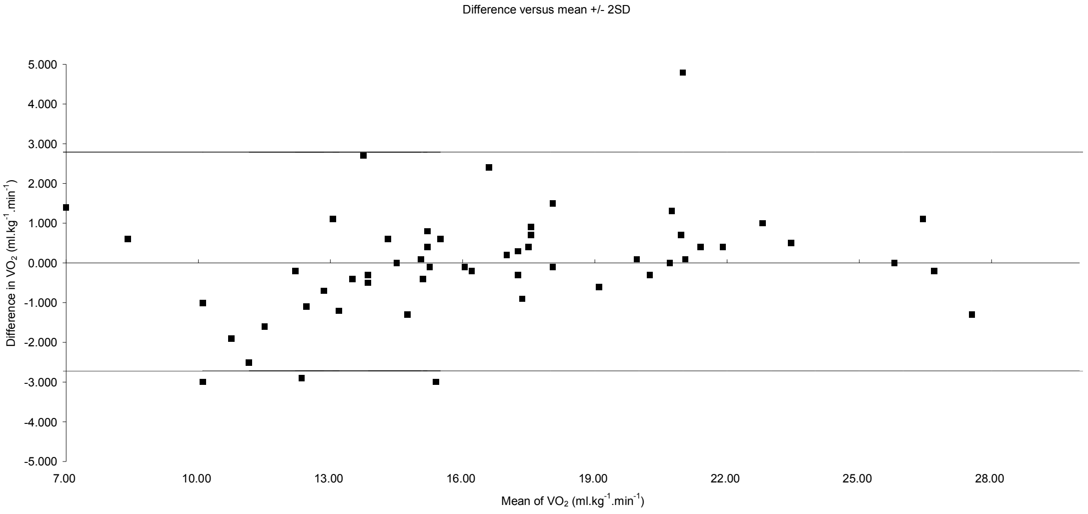
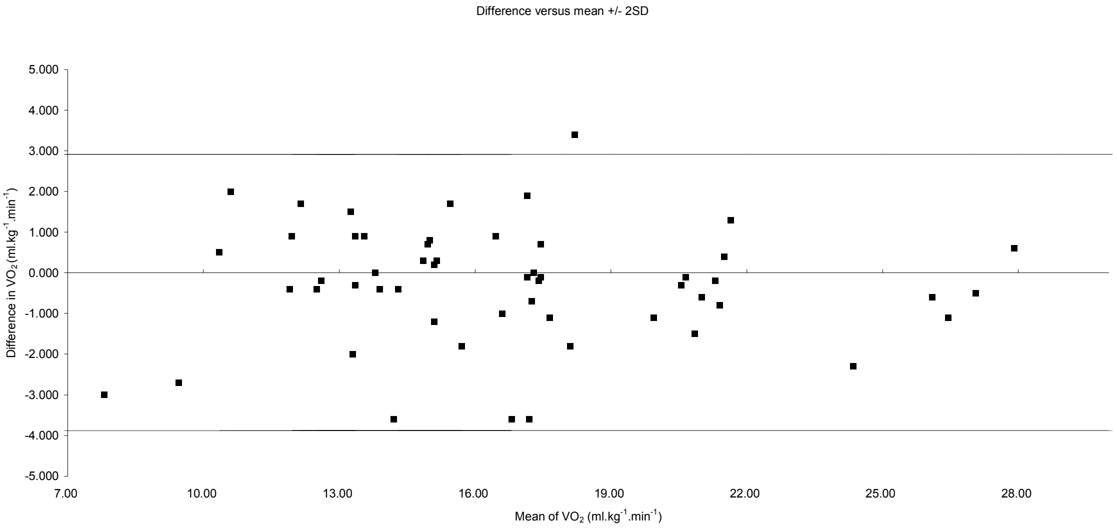


Figure 3.4 – Between-day difference in $\dot{V}O_2$ (ml.kg^{-1}.min^{-1})



Individual within- and between-day CV ranged from 0% to 16%. Group variability was also measured as CV in percentages. There was a lower $\dot{V}O_2$ variability for 0.8 km.h^{-1} less than SS and for SS compared with the slower and faster speed in both within-and between-day analyses. However, there were no statistically significant differences in within- or between-day group CV at any speed for $\dot{V}O_2$. There were also no statistically significant differences in within- or between-day CV for HR and

GE at any speed. Within and between-day mean CV's by speed for $\dot{V}O_2$, GE and HR are shown in Table 3.3.

Table 3.2 Mean difference, 95% CI and standard error of measurement (SEM) for within and between-day $\dot{V}O_2$, GE and HR per speed.

	V1		***V2***	
	WD	BD	WD	BD
$\dot{V}O_2$				
Mean difference	-0.14	-0.36	-0.27	-0.31
95% CI	-0.95 to 0.68	-1.38 to 0.66	-0.89 to 0.35	-1.02 to 0.39
SEM	0.38	0.47	0.29	0.33
GE				
Mean difference	-0.003	-0.006	-0.004	-0.005
95% CI	0.018 to 0.012	-0.025 to 0.012	-0.013 to 0.005	-0.015 to 0.006
SEM	0.007	0.008	0.004	0.005
HR				
Mean difference	-1.64	-0.29	-1.57	-0.57
95% CI	-4.32 to 1.03	-4.55 to 3.98	-4.09 to 0.95	-4.87 to 3.73
SEM	1.24	1.97	1.17	1.99

	SS		***V4***	
	WD	BD	WD	BD
$\dot{V}O_2$				
Mean difference	0.32	-0.57	0.24	-0.68
95% CI	-0.40 to 1.04	-1.36 to 0.22	-0.82 to 1.30	-2.11 to 0.75
SEM	0.33	0.36	0.49	0.66
GE				
Mean difference	0.004	-0.007	0.003	-0.007
95% CI	-0.005 to 0.013	-0.017 to 0.003	-0.008 to 0.014	-0.022 to 0.008
SEM	0.004	0.005	0.005	0.007
HR				
Mean difference	-1.00	0.14	-1.16	-0.29
95% CI	-3.73 to 1.73	-3.67 to 3.95	-4.99 to 2.67	-4.98 to 4.41
SEM	1.26	1.76	1.77	2.17

Table 3.3- Within- and between-day mean CV's for $\dot{V}O_2$*, HR and GE per speed by test*

Test	**< 1.6 km.h^{-1}**			**< 0.8 km.h^{-1}**			**SS**			**> 0.8 km.h^{-1}**		
	$\dot{V}O_2$ (%)	HR (%)	GE (%)	$\dot{V}O_2$ (%)	HR (%)	GE (%)	$\dot{V}O_2$ (%)	HR (%)	GE (%)	$\dot{V}O_2$ (%)	HR (%)	GE (%)
WD	7.3	2.1	7.4	3.6	2.1	4.9	3.8	1.9	4.2	4.3	2.4	4.6
BD	7.9	3.6	7.9	5.1	3.1	5.6	4.4	3.1	4.7	5.5	3.0	6.7

WD = within-day; BD = between-day; $\dot{V}O2$ *= oxygen uptake; HR = Heart rate; GE = gross economy;SS = self-selected speed*

3.3.4 Discussion

The range of walking speeds used in the present study was similar to other investigations in young children using the mean of the individual's "comfortable walking pace" (Frost et al., 1995; Tseh et al., 2000; Morgan et al., 2004). This speed was deemed by Skinner et al. (1971) to be the appropriate speed for obtaining standardised submaximal $\dot{V}O_2$ data in children aged 6 to 7.9 years. According to Sutherland (1997), at 6 to 7 years of age children assume all the electromyographic, kinematic, and kinetic walking characteristics of an adult. Similarly, Jeng et al. (1997) verified that children younger than seven years tend to display difficulties in modulating walking steps on the treadmill while 7- to 12 year-old children demonstrate walking features comparable with adults.

Another important consideration regarding walking speed determination is that the study by Frost et al. (1995) is the only one other than ours to have used a comfortable walking pace over-ground to determine the treadmill speed, however there were differences in both over-ground and treadmill protocols. An earlier observation also showed that where speed and step rate (or step length) of walking are specified this constitutes a forced walk and the energy expenditure under such conditions could be different from the free or self-selected walking speed (Zarrugh et al., 1974). These assertions make comparisons between studies using different techniques to assess preferred and forced speed problematic as biases could be introduced.

The methodological approach used in our study to determine the self-selected speed resulted in a mean speed higher than 1.39 $m.sec^{-1}$ reported in adolescents with a mean age of 17.3 years (Wergel-Kolmert & Wohlfart, 1999). In contrast, the mean speed in the present study for children with a mean age of 10.1 years was the same as the self-selected speed found for normal weight women (Browning et al., 2006). From the age of 7 years, the kinematic curves are identical to those of an adult and only the temporal parameters change in direct relation to the length of the limbs. Puberty further disturbs these linear relations and the influence of skeletal growth may be most marked in relation to movement speed (Norlin, Odenrick, & Sandlund, 1981). These considerations should privilege the older and more mature participants with higher self-selected walking speeds than our younger subjects. The inconsistencies regarding self-selected walking speed among studies in different age categories, including differences in protocol makes it difficult to produce a standardised definition or more distinct understanding regarding the meaning of "comfortable or self-selected walk speed".

It is interesting to note the way in which trials to verify the reproducibility of $\dot{V}O_2$ have been conducted. Within-day studies have typically used a different number of bouts across the same exercise session and a rest period between each trial considering time (Tseh et al., 2000; Keefer et al., 2005) and/or heart rate recovery conditions (Maltais et al., 2003). In the present study children underwent 2 different exercise sessions on the same day showing repeatability as can be seen when comparing individual measurements against their means from both exercise sessions (Table 3.2). On the other hand, between-day studies have varied greatly in the times between trials including 2 consecutive days (Wergel-Kolmert & Wohlfart, 1999), 5 days maximum interval (Frost et al., 1995), 1 week interval (Tseh et al., 2000; Keefer et al., 2005) and 2 weeks maximum interval (Maltais et al., 2003). The only studies to consider the time of the day between tests to control for circadian variation were Frost et al. (1995) and Keefer et al. (2005). Our between-day analyses with 1-week interval between sessions showed $\dot{V}O_2$ to be repeatable considering the same criteria used for within-day analyses, assessed at 4 different walk speeds.

Within-day $\dot{V}O_2$ CV showed greater variability at lower (7.3%) and higher speeds (4.3%). The SS and 0.8 $km.h^{-1}$ less than SS evidenced the lowest CV (3.6% and 3.8%). These results elicited a U-shaped curve among CV by speed in the same way that can be seen when GE is plotted versus walking speed (Figure 3.2). These results demonstrate the lower variability in $\dot{V}O_2$ when children walk at a comfortable speed (SS) or at near lower velocity (0.8 $km.h^{-1}$ less than SS).

The within-day $\dot{V}O_2$ CV at SS in the present study is somewhat similar but a little higher that the results of Tseh et al. (2000). This difference could be attributable to the alternative approaches to analyse within-day variability in our study using tests on two different periods of time of the day against the data collection in the same session in the cited study. On the other hand our between-day $\dot{V}O_2$ CV at SS was lower than the CV of 7.5% for individual pace and 6.4% for a forced 5 km.h^{-1} (Wergel-Kolmert & Wohlfart, 1999).

The SS determined over-ground and its ability to verify the preferred walking speed on the treadmill showed a reproducible $\dot{V}O_2$ within-and between-day considering all derived speeds. The measurement of SS over-ground was undertaken to reflect the speed an individual would select during the common walking tasks of everyday life (Browning & Kram, 2005) as well as from the perspective of exercise prescription. The reliability of the oxygen uptake in children walking at a comfortable speed could be an important indicator for training control because most activities of daily living represent exertion at a submaximal exercise capacity and training effects under such conditions may result in limited (Watts, Jones, Davis, & Green, 2005), or no change in maximal capacity. The SS could be a useful starting point for exercise prescription and further increases in intensity based on this starting speed could be easily assimilated.

HR showed lower variability within and between-day at all velocities (Table 2). The lowest HR variability between-day at submaximal treadmill walking was previously reported (Frost et al., 1995). Formulae to estimate maximal heart rate are inappropriate for children however children's walking protocols usually elicit a peak rate of approximately 195 bpm (ACSM, 2006). The ACSM (2006) postulate that any aerobic training adaptations that occur in children can be elicited by the same frequency and duration of training criteria as those recommended in adult programs. As $\dot{V}O_2$ max measurements were not taken, we cannot verify the percentage maximum HR in this study. However healthy children do not generally require heart rate monitoring in daily exercise sessions because of their low cardiac risk and their ability to adjust exercise intensity through rating of perceived exertion (ACSM, 2006), mainly during sub- maximal exercise (Robertson et al., 2000; Utter, Robertson, Nieman, & Kang, 2002).

A familiarisation protocol based on the SS of children could be enough to achieve accommodation to the treadmill and measure reliable within- and between-day $\dot{V}O_2$ in children performing treadmill exercise. The observed within- and between-day CV showed lower variability at a comfortable speed (SS) or at near the lower velocity (0.8 km.h^{-1} less than SS). Repeatability within and between-day for $\dot{V}O_2$, GE and HR for all speeds was verified. These results suggest that submaximal $\dot{V}O_2$ during treadmill walking is stable and reproducible at a range of speeds based on children's SS.

Physical activity programs for children should emphasise exercise behaviours rather than outcomes with intensive training to improve physical conditioning withheld until after puberty. The physiological cost of walking is an important determinant of functional capacity and related daily physical activities. Increasing energy expenditure by changing lifestyle activity patterns provides an alternative to programmed exercise training regimens for children and may be better tolerated and maintained because each exercise change is smaller and easier to manage. For most children, walking is the major physical activity of daily life. It is one of the most common, easy and cheap ways to increase daily energy expenditure in recreation, transportation or physical activity.

3.4 Substrate oxidation pattern in girls: Self-selected speed for exercise control.

Modified from: Amorim PRS, Byrne NM, Hills AP (paper under review) 'Substrate oxidation pattern in girls: Self-selected speed for exercise control.'

3.4.1 Introduction

Fat and carbohydrate (CHO) are the principal substrates that fuel aerobic ATP synthesis in human skeletal muscle. The relative utilization of fat and CHO during exercise can vary enormously and largely depends on exercise intensity (van Loon, Greenhaff, Constantin-Teodosiu, Saris, & Wagenmakers, 2001).

Obesity is reported to be associated with an impaired ability to use fat as a fuel and this may contribute to the development and maintenance of larger fat stores (van Aggel-Leijssen et al., 2002). Fat balance includes trafficking of fat to tissues (liver, muscle, adipose), and the subsequent partitioning of fat to oxidation or storage. Positively altering the trafficking and partitioning of fat may help to determine how individuals are able to maintain body weight and body composition (Hansen et al., 2005).

Indirect calorimetry (IC) can provide information on the type and rate of fuel oxidation within the body (Frayn, 1983), including the determination of the composition of the mixture of CHO and fat oxidized (Peronnet & Massicotte, 1991). A fundamental assumption in the determination of fat and CHO utilization using IC is that $\dot{V}O_2$ and $\dot{V}CO_2$ measured in expired air reflects $\dot{V}O_2$ and $\dot{V}CO_2$ at the tissue level. Whereas $\dot{V}O_2$ will reliably reflect the tissue $\dot{V}O_2$, $\dot{V}CO_2$ is only a reliable estimate of tissue $\dot{V}CO_2$ in the presence of a stable bicarbonate pool (Venables, Achten, & Jeukendrup, 2005). The determination of exercise IC appears to be valid for the measurement of substrate oxidation during submaximal steady-state exercise bouts (Brandou, Dumortier, Garandeau, Mercier, & Brun, 2003).

There is general consensus in the literature that shifts in energy substrate mobilization and utilization occur as exercise intensity increases. The pattern of substrate utilization in an individual at any point in time depends in part on the crossover between the exercise intensity-induced responses (which increase CHO utilization) and the endurance training-induced responses (which promote lipid oxidation). The crossover point is identified as the power output at which energy derived from the oxidation of CHO-based fuels predominates over that derived from lipids, with further increases in power eliciting a relative increment in energy from CHO utilization and a relative decrement in energy from lipid oxidation (Brooks & Mercier, 1994).

To verify the effects of exercise intensity on habitual substrate utilization, a range of modalities of exercise have commonly been employed. Modalities have included cycling using ergometers (Achten et al., 2002; van Aggel-Leijssen et al., 2002; Votruba, Atkinson, Hirvonen, & Schoeller, 2002; Timmons, Bar-Or, & Riddell, 2003; Zunquin, Theunynck, Sesboue, Arhan, & Bougle, 2006), running (Martinez & Haymes, 1992; Venables et al., 2005) or walking on the treadmill with forced velocity (Deriaz, Dumont, Bergeron, Despres, Brochu, & Prud'homme, 2001; Maffeis et al., 2005), and with exercise intensity expressed as a percentage of maximal (or peak) oxygen uptake.

To date, no data are available on the relationship between substrate utilization and exercise intensity expressed as velocity based on the self-selected walking speed in girls. We chose to perform this study in normal weight girls to establish the effect of walking speeds on substrate oxidation during a treadmill protocol based on SS.

3.4.2 Materials and Methods

3.4.2.1 Participants

Twelve girls aged 8-12 (mean age = 9.9±1.4 yr, mass = 34.1±6.9 kg, height = 143.3±10.9 cm) participated in the study. The study protocol was approved by the Human Research Ethics Committee at the Queensland University of Technology and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Participants and their parents or caregivers gave written informed consent prior to inclusion in the study.

3.4.2.2 Study Design

The self-selected speed (SS) determination and treadmill accommodation were performed using the same procedure described earlier in session 3.2.2.2.

Participants were familiarized with the OMNI-walk/run scale for the rating of perceived exertion (RPE). The children's OMNI-walk/run scale contains both pictorial and verbal descriptors positioned along a comparatively narrow numerical response range of 0 to 10 validated to assess RPE during treadmill exercise in children aged 6 to 13 years (Utter et al., 2002). Each participant underwent 2 test sessions, one week apart. Sessions were conducted between 7:00 and 8:30am and participants were instructed to remain in an overnight fasted state.

3.4.2.3 Data Collection

The treadmill protocol was the same as described at session 3.3.2.3. The OMNI scale was administered in the last 30 secs for each speed. Participants were fitted with a nose and mouth pediatric face mask (FM) (series model 8950 - Hans Rudolph, Inc.) with a two-way breathing T-shaped valve and pneumotach. To improve the seal between face and mask an Ultimate Seal^{TM} gel was used. Participants were also fitted with a Polar transmitter (Polar WearLink Transmitter, Polar Electro Oy, Finland) and a receiver (model S610 Polar Electro Oy, Finland). Ventilation (V_E), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were collected as breath-by-breath samples using a MOXUS Modular $\dot{V}O_2$ System. A turbine attached to the inspiration side of a Hans Rudolph two-way non-rebreathing valve measured volume and airflow, and it was verified before each measurement. Oxygen concentration of expiration was measured via an S-3A Oxygen Analyser sensor that contained a stabilised zirconia high-temperature electrochemical cell with an inherently rapid response to changes in oxygen concentrations from 0-100%. Carbon dioxide concentration of expiration was measured via a CD-3A Carbon Dioxide Analyser which contained infrared optics with filters, a chopper and a cooled lead selenide detector with preamplifier capable of continuous and accurate analysis of carbon dioxide concentrations from 0-15%. A Model R-1 Flow Control that contained a flow meter, pump and needle valve was responsible for drawing the sample gases through the sensors and then venting them to the room. Oxygen and carbon dioxide gas analysers were calibrated before each measurement using known standard gas concentrations.

3.4.2.4 Data analyses

A preliminary stabilisation period of 3 min was allowed for each speed followed by a 3 min sampling period. From respiratory measurements, total fat and carbohydrate (CHO) oxidation rates were calculated according to the non-protein respiratory quotient (RQ) (Peronnet & Massicotte, 1991):

$$\text{CHO oxidation rate} = 4.585\,\dot{V}CO_2 - 3.226\,\dot{V}O_2$$

$$\text{Fat oxidation rate} = 1.695\,\dot{V}O_2 - 1.701\,\dot{V}CO_2,$$

with $\dot{V}O_2$ and $\dot{V}CO_2$ in litres per minute ($l.min^{-1}$) and oxidation rates in grams per minute ($g.min^{-1}$).

The percentage of CHO and fat oxidation were calculated using the following equations (McGilvery & Goldstein, 1983): $\%CHO = ((RQ - 0.71)/0.29) \times 100$

$$\%Fat = ((1 - RQ)/0.29) \times 100$$

To estimate the percentage of maximal oxygen consumption from relative submaximal heart rate the mathematical regression model proposed by Londeree and Ames (Londeree & Ames, 1976) was used.

Repeated measures ANOVA followed by a *Tukey Post Hoc* test at a significance level of $p<0.05$ was used to verify differences in CHO and fat oxidation rates among speeds. Paired T-test was used to verify differences in CHO and fat oxidation rates between tests per velocity. The reliability of the $\dot{V}O_2$, $\dot{V}CO_2$, CHO and fat absolute ($g.min^{-1}$) and relative ($g.kg^{-1}.h^{-1}$) between–day were assessed using intraclass correlation coefficients (ICC). The coefficient of variation (CV) was calculated for heart rate (HR) per velocity.

3.4.3 Results

The range of preferred speed measured over-ground and used to derive all other velocities on the treadmill was between 4.5 and 6.4 $km.h^{-1}$. (Mean preferred speed = 5.3 ± 0.6 $km.h^{-1}$). In this study it was important that the measurement of $\dot{V}O_2$ was undertaken with participants in a steady state. Using the criterion for steady-state

(Frost et al., 1995), no participants differed by more than 2 $ml.kg^{-1}.min^{-1}$ in $\dot{V}O_2$ between the last 2 min in any 6 min exercise bout.

Results from the repeated measures ANOVA showed significant differences for CHO oxidation among all speeds, as well as significant differences for fat oxidation between V1 and V2 against V3 and V4 in both tests. Paired T-test between trials per velocity showed no significant substrate use differences in any velocity between days. Absolute and relative CHO and fat oxidation per velocity can be seen in Table 3.4.

Table 3.4 - Absolute and relative CHO and FAT oxidation per velocity by test

Fat	Absolute ($g.min^{-1}$)		Relative ($g.kg^{-1}.h^{-1}$)	
	T1	T2	T1	T2
V1	0.14±0.03	0.16±0.04	0.26±0.08	0.28±0.09
V2	0.16±0.04	0.16±0.06	0.29±0.10	0.30±0.11
V3	0.18±0.04	0.19±0.05	0.33±0.10	0.34±0.12
V4	0.19±0.04	0.19±0.06	0.35±0.11	0.35±0.14
CHO				
V1	0.18±0.08	0.18±0.10	0.31±0.11	0.31±0.15
V2	0.21±0.09	0.24±0.14	0.36±0.13	0.40±0.20
V3	0.29±0.14	0.30±0.15	0.51±0.20	0.51±0.24
V4	0.42±0.21	0.45±0.25	0.73±0.29	0.78±0.37

Figure 3.5 presents the relationship between energy derived from CHO and fat and exercise intensity, expressed as velocity. Fat oxidation reduced and CHO increased as exercise intensity increased.

Average values and standard deviation for $\dot{V}O_2$, $\dot{V}CO_2$, RQ, HR and OMNI per velocity by test are shown in Table 3.5, as well as CV for HR and RQ range by velocity.

The intraclass correlation coefficient (ICC) for between-day $\dot{V}O_2$, $\dot{V}CO_2$, CHO ($g.min^{-1}$), fat ($g.min^{-1}$), CHO ($g.kg^{-1}.h^{-1}$) and fat ($g.kg^{-1}.h^{-1}$) were 0.98, 0.97, 0.79, 0.60, 0.71 and 0.72, respectively.

Figure 3.5 - CHO and FAT oxidation per velocity by test (%)

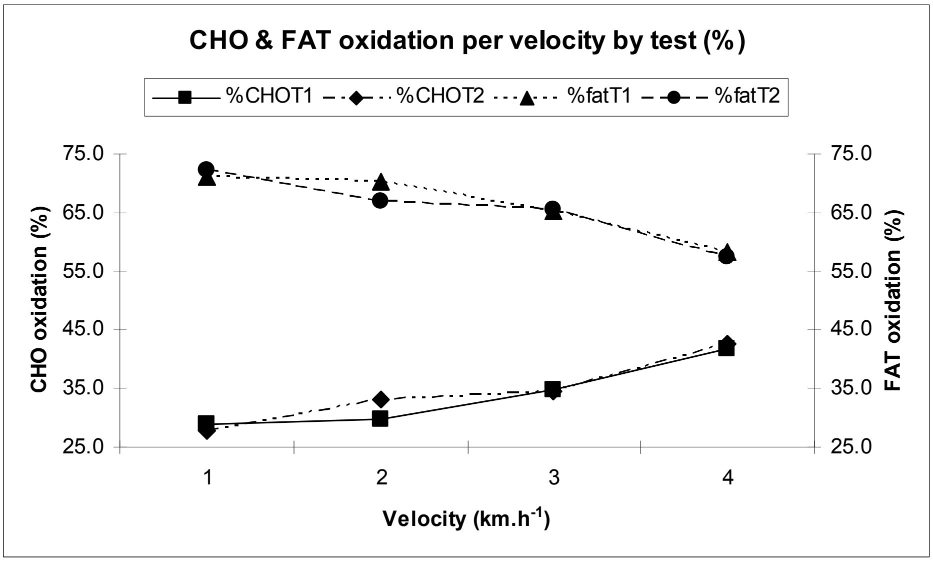


Table 3.5 - $\dot{V}O_2$ (l.min^{-1}), $\dot{V}CO_2$ (l.min^{-1}), RQ, HR (bpm) and OMNI per test and speed

	T1	T2
$\dot{V}O_2$		
V1	0.43±0.10	0.45±0.09
V2	0.48±0.12	0.51±0.10
V3	0.58±0.15	0.60±0.13
V4	0.70±0.19	0.73±0.17
$\dot{V}CO_2$		
V1	0.34±0.08	0.35±0.07
V2	0.38±0.10	0.41±0.09
V3	0.47±0.13	0.48±0.11
V4	0.59±0.17	0.61±0.17
RQ		
V1	0.79±0.03 (0.75 to 0.85)$^{\wedge}$	0.79±0.05 (0.74 to 0.90)$^{\wedge}$
V2	0.80±0.03 (0.75 to 0.85$^{\wedge}$	0.81±0.06 (0.74 to 0.93)$^{\wedge}$
V3	0.81±0.03 (0.77 to 0.87)$^{\wedge}$	0.81±0.05 (0.74 to 0.91)$^{\wedge}$
V4	0.83±0.04 (0.79 to 0.87)$^{\wedge}$	0.83±0.06 (0.74 to 0.94)$^{\wedge}$
HR		
V1	108±13 (0.03)*	110±15
V2	113±15 (0.02)*	116±16
V3	122±17 (0.03)*	124±14
V4	137±20 (0.02)*	139±19
OMNI		
V1	1.0±0.7	0.5±0.7
V2	1.8±0.9	1.3±1.3
V3	3.1±1.2	2.6±1.4
V4	4.4±1.4	4.1±1.9

$^{\wedge}$RQ range * HR CV between T1 and T2

3.4.4 Discussion

As expected, the absolute rates of CHO oxidation continued to rise with the increased exercise intensity and the greatest use occurred during the most intense exercise. It must be noted that the absolute rates of fat oxidation are dependent on CHO intake (Achten et al., 2002) consequently affecting the contribution of CHO to total energy expenditure. To prevent this influence on oxidation rates, all exercise tests in this study were performed in a fasted state of at least 12 hours.

Fat oxidation started to increase from V1 to V3, and achieved a plateau at this velocity as indicated by no significant fat oxidation differences between V3 and V4. The verified fat oxidation difference, expressed as absolute or relative, between the 2 slow velocities (V1 and V2) and 2 fast velocities (V3 and V4) with the greatest fat oxidation in the two last, without significant difference between them, could indicate that the self-selected speed (V3) promotes higher fat oxidation rates than more intense exercise, which does not add any advantage in terms of fuel utilization. Figure 3.5 outlines the relative fuel contribution from CHO and fat where an increased CHO and decreased fat oxidation after the self-selected speed can be verified. It is relevant to acknowledge that the reproducibility expressed as ICC was acceptable and statistically significant for $\dot{V}O_2$, $\dot{V}CO_2$, and absolute and relative CHO and fat oxidation.

One could argue that the difference could be attributed to not reaching an acid-base balance that habitually occurs at higher exercise intensities and, consequently, this introduced limitations in the ability of IC to determine substrate utilization accurately under this condition. In general the ventilatory anaerobic threshold of children occurs at a higher percentage of $\dot{V}O_2$ max than in adults, decreases with age and is lower in girls than in boys (Nixon, 2000). The average intensity achieved in the two faster velocities (V3 and V4) measured as maximal heart rate percentage was 64±9% and 70±10% which are equivalent to 50% and 55% of $\dot{V}O_2$ max. These values are below the higher ventilatory or lactate thresholds of 58% to 83% of $\dot{V}O_2$ max reported in the pediatric literature (Atomi, Iwaoka, Hatta, Miyashita, & Yamamoto, 1986; Rotstein, Dotan, Bar-Or, & Tenenbaum, 1986; Washington, 1989). The measurement of respiratory quotient (RQ) in our study may help dismiss any doubt regarding the stable acid-base balance achieved by participants in both tests. At the fast velocity both tests showed an average RQ of 0.83±0.06 with a range from 0.74 to 0.94. However, comparisons between IC and more stringent techniques have showed that absolute substrate oxidation could be determined independently of a stable bicarbonate pool (Romijn, Coyle, Hibbert, & Wolfe, 1992).

To the best of our knowledge only one previous study has verified the relationship between the absolute and relative nutrient oxidation and walking speed (Maffeis et al., 2005). When we compare our results for self-selected speed in girls (5.3 $km.h^{-1}$) with the second velocity (5 $km.h^{-1}$) analysed in the Maffeis study, fat oxidation ($g.mim^{-1}$) and $\dot{V}O_2$ max percentage were similar, but CHO oxidation ($g.min^{-1}$) in our study was lower. We can attribute the higher CHO oxidation in the previous study to two important methodological differences. The first was that we took measurements in the fasting state, whereas Maffeis et al. tested under post-absorptive conditions. The variability due to nutritional status (fed versus fasted) may have been significant. To support this premise there was a higher RQ in the Maffeis et al. study (0.87±0.05) compared with the present study RQ (0.81±0.05), despite a little higher average velocity in our study. Secondly, we used a non-protein RQ whereas the previous study used an estimate of protein oxidation. The non-protein RQ approach, widely used today, assumes that the amount of protein oxidized is negligible and that other metabolic processes, which involve the production and/or utilization of O_2 and/or CO_2 are also quantitatively negligible compared to glucose and fatty acid oxidation (Peronnet & Massicotte, 1991).

There is some contradictory evidence regarding whether women oxidise fat preferentially during exercise. However, collectively the evidence suggests that women oxidise proportionately more lipid compared with males (Hansen et al., 2005). The extent to which this evidence could be applied to children is not known and future studies including both boys and girls are warranted to determine possible gender differences in substrate metabolism.

Despite the methodological differences and somewhat different results, we are in agreement with Maffeis et al. (2005) regarding walking speed performed at low to moderate intensity being feasible for any able-bodied children, whether or not they are obese. Our strategy to use derived velocities based on the self-selected walking speed was chosen to provide a more appropriate basis for comparison. We wanted exercise intensities to be relative to perceived exertion and to use a reference workload that was applicable in free-living conditions. Further, our average results from the OMNI scale of perceived exertion showed that at the self-selected speed a value of “3”, a number between the perceptual anchors of “a little tired - 2” and “getting more tired – 4”, could be classified as a moderate effort. The less intense exercise is unlikely to be followed by compensatory sedentary behaviour, as may be the case for more strenuous exercise (Maffeis et al., 2005).

The most important finding of this study was the relationship between the relative energy derived from CHO and fat and walking velocities (Figure 3.5). At the self-selected speed (V3) there was an accentuation in fat oxidation reduction as well as an increase in CHO oxidation. Our range of speeds was not sufficient to verify the crossover of fuel utilization and this is an acknowledged study limitation. The “crossover concept” of fuel utilization occurs at approximately 50% of $\dot{V}O_2$ max (Brooks & Mercier, 1994), varying between 48 and 53% (Venables et al., 2005). Despite our inability to verify the crossover point, our sample showed an average HR at the self-selected speed of 64% of HR max (range = 46 to 73% HR max), which represents 50% of $\dot{V}O_2$ max.

Considering an RQ higher than 0.85 as an index of fat to carbohydrate oxidation ratio, and the verified RQ at self-selected speed (mean: 0.81, range: 0.74 to 0.91) associated with the verified plateau in fat oxidation, we believe that these findings on the whole are indicative that this speed should be the starting point of an inversion of fuel oxidation. Further, this speed may be an upper cut-off point for intensity control during exercise for girls to optimize fat utilization during exercise.

Exercise prescription is a mix of both art and science where the “tools” used to control intensity are the keys to the desired outcomes. Indicators of exercise intensity that can be applied in daily practice outside the laboratory setting include speed (mechanical indicator), heart rate (physiological indicator) and RPE (subjective indicator). Our results need to be replicated in participants of other ages and at different speeds, but in the light of the battle to control the advance of obesity in children we believe that moderate intensity, expressed here as a velocity approximating the self-selected speed, could be an “upper cut-off point” to control exercise intensity and optimize fat oxidation in girls (mechanical indicator). To facilitate such applicability, the self-selected speed could be measured in a standardized fashion and then parents or children taught to verify whether the target speed was subsequently achieved during day-to-day practice. To oversee this it is only necessary to know the exercise time and distance. To further refine exercise control, the use of other indicators such as percentage of maximal heart rate below 64% (or below 50% of $\dot{V}O_2$ max) (physiological indicator) and OMNI scale of perceived exertion below “3” (subjective indicator) could be of additional value. The combined use of all indicators should be reinforced as the preferred option.

Weight gain for many individuals appears to result from small additive effects that occur periodically throughout our daily lives (Hansen et al., 2005). As the prospect of long-term weight loss continues to elude most people, the prevention of further weight gain assumes greater importance (Votruba et al., 2002). Contemporaneously, perhaps one of the most urgent actions is to facilitate active lifestyles. Walking is a natural, accessible and safe physical activity and does not require special ability or equipment. For most individuals walking is the key activity of daily life. A logical starting point for children could be the determination and use of the self-selected speed and the subsequent introduction of other indicators as a function of acceptance, adherence and the results gained with the intervention procedure.

3.5 Comparison of substrate oxidation and body-size related performance in girls and women during treadmill walking.

Modified from: Amorim PRS, Byrne NM, Hills AP (paper under review) 'Comparison of substrate oxidation and body-size related performance in girls and women during treadmill walking.'

3.5.1 Introduction

A range of exercise modalities have been employed to assess the effects of exercise intensity on habitual substrate utilization, including cycle ergometers (Achten et al., 2002; van Aggel-Leijssen et al., 2002; Votruba et al., 2002; Timmons et al., 2003; Zunquin et al., 2006), and running (Martinez & Haymes, 1992; Venables et al., 2005) or walking on a treadmill with forced velocity (Deriaz et al., 2001; Maffeis et al., 2005). Commonly, exercise intensity is expressed as a percentage of maximal (or peak) oxygen uptake and comparisons of substrate oxidation at fixed percentages of $\dot{V}O_{2max}$ are undertaken to 'normalise' for level of cardiorespiratory fitness. While this approach has merit, it is based on three important assumptions or requirements. Firstly, it requires that $\dot{V}O_{2max}$ be measured; an imposition that can limit the size and characteristics of the sample that can be recruited. Secondly, it requires that $\dot{V}O_{2max}$ can be accurately measured; this can be more difficult in a younger cohort. Finally, there is the assumption that work undertaken at a proportion of maximal oxygen uptake reflects the same physiological stress on the body for all individuals. We (Byrne & Hills, 2002) and others (Dwyer & Bybee, 1983; Weltman, Snead, Seip, Schurrer, Weltman, Rutt, & Rogol, 1990; Meyer, Gabriel, & Kindermann, 1999) have shown that the same relative intensity corresponds to wide ranges of exercise intensity as defined relative to the lactate threshold (for example, 50% $\dot{V}O_{2max}$ corresponded to 38-88% of the lactate threshold). Therefore, the equivalence of a specific relative proportion of maximal aerobic power in terms of substrate oxidation may be questioned.

Self-selected walking speed is commonly identified as the most efficient walking speed; with increased efficiency defined by lower $\dot{V}O_2$ per unit mechanical work (Hoyt & Taylor, 1981; Taylor et al., 1982; Hreljac, 1993). It may be argued that a more valid and reliable analysis of differences in the energy cost and substrate utilization of different cohorts when exercising may be possible when employing speeds greater than and less than this reproducible reference. To the best of our knowledge, no study to date has explored the relationship between substrate utilization and exercise intensity expressed as velocity based on the self-selected walking speed in girls and women.

Studies using the conventional ratio standard for body mass to compare children and adults have been criticised (Katch, 1973; Katch & Katch, 1974; Welsman et al., 1996; Welsman & Armstrong, 2000). The process of "scaling" has been used as an alternative approach to compare individuals with different body mass in an attempt to control for the structural and functional consequences of changes in size or scale among otherwise similar organisms (Schmidt-Nielsen, 1984). There is still considerable debate in both the adult and paediatric literature concerning the numeric value of the mass exponent and whether there is a 'true" exponent that might provide a universal alternative to simple per body mass scaling (Welsman & Armstrong, 2000).

The objective of the current study was to determine whether there are differences in oxygen uptake as well as substrate oxidation between girls and women during a treadmill protocol based on self-selected walking speed.

3.5.2 Materials and Methods

3.5.2.1 Participants

Experiments were carried out on 12 girls (G) aged 8-12 (mean values: age = 9.9±1.4 yr, mass = 34.1±6.9 kg, height = 143.3±0.1 cm) and 12 women (W) aged 25-38 (mean values: age = 32.3±3.8 yr, mass = 63.2±9.4 kg, height = 170.0±0.1 cm, BMI = 21.9±2.5 kg.m^{-2}). Girls were recruited from the Brisbane metropolitan area through advertisements distributed in schools. Data for women (BMI < 25kg/m^{2}) were derived from a larger project conducted by our group on the energy cost of self-paced walking in adult men and women of varying body size and composition. All participants were in good health and were not taking medications known to influence metabolism. The study protocol was approved by the Human Research Ethics Committee at the Queensland University of Technology and performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Participants and parents or caregivers of the children gave written informed consent prior to their inclusion in the study.

3.5.2.2 Study Design

On the morning of the first visit participants arrived at the exercise physiology laboratory by car having been instructed to remain in an overnight fasted state (no food or drink, except for water) and having abstained from moderate and strenuous exercise in the previous 24 hours. For resting metabolic rate (RMR) measurements participants were fitted with a silicone mouthpiece (standard type - Hans Rudolph, Inc.) and a disposable nose-clip (model 9014 - plastic and foam - Hans Rudolph, Inc.) and data collection took place in a thermo-regulated environment. A preliminary stabilisation period of 20 min was allowed followed by a 30 min measurement period with participants lying and monitored periodically to ensure that they remained awake. The self-selected speed (SS) determination and treadmill accommodation were performed using the same procedure described early at session 3.2.2.2 for girls. Walking speed data for the women was determined using a protocol as previously described (Hills et al., 2006). Similarly, girls were familiarized with the OMNI scale for the rating of perceived exertion (Utter et al., 2002) and women with the Borg Scale (Borg, 1970).

3.5.2.3 Data Collection

The treadmill protocol was the same as described in session 3.3.2.3. The OMNI or Borg scale was administered in the last 30 sec for each speed. Participants were fitted with a heart rate monitor (Polar Electro Oy, Kempele, Finland). Ventilation (V_E), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were assessed from breath-by-breath samples using a MOXUS Modular $\dot{V}O_2$ System. A turbine attached to the inspired side of a Hans Rudolph mouthpiece measured volume and airflow, and it was verified before each measurement using a certified 3-Litre calibration syringe. Verification was achieved when volumes at 60 L/min, 90 L/min and 120 L/min were within ± 1.5% of syringe volume. Oxygen concentration of expiration was measured via an S-3A Oxygen Analyser sensor that contains a stabilised zirconia high-temperature electrochemical cell which has an inherently rapid response to changes in oxygen concentrations from 0-100%. Carbon dioxide concentration of expiration was measured via a CD-3A Carbon Dioxide Analyser which contained infrared optics with filters, a chopper and a cooled lead selenide detector with preamplifier capable of continuous and accurate analysis of carbon dioxide concentrations from 0-15%. A Model R-1 Flow Control that contained a flow meter, pump and needle valve was responsible for drawing the sample gases through the sensors and then venting them to the room. Oxygen and carbon dioxide gas analysers were calibrated before each measurement using known standard gas concentrations (4.03% CO_2, 15.10% O_2).

3.5.2.4 Data Analyses

A preliminary stabilisation period of 3 min was allowed for each speed followed by a 3 min sampling period. From respiratory measurements, total fat and carbohydrate (CHO) oxidation rates were calculated according to the non-protein respiratory quotient (RQ) (Peronnet & Massicotte, 1991):

$$\text{CHO oxidation rate} = 4.585\,\dot{V}CO_2 - 3.226\,\dot{V}O_2$$

$$\text{Fat oxidation rate} = 1.695\,\dot{V}O_2 - 1.701\,\dot{V}CO_2,$$

with $\dot{V}O_2$ and $\dot{V}CO_2$ in litres per minute ($l.min^{-1}$) and oxidation rates in grams per minute ($g.min^{-1}$).

The percentage of CHO and fat oxidation were calculated using the following equations (McGilvery & Goldstein, 1983): $\%CHO = ((RQ - 0.71)/0.29) \times 100$

$\%Fat = ((1 - RQ)/0.29) \times 100$

To estimate the percentage of maximal oxygen consumption for girls from relative submaximal heart rate, the mathematical regression model proposed by Londeree and Ames (1976) was used.

For between-group comparisons, due to the controversy in the literature regarding the best scaling method, $\dot{V}O_2$ was expressed in different ways as following: absolute values ($L.min^{-1}$), simple ratio with body mass ($ml.kg^{-1}.min^{-1}$), body surface area ($ml.m^{-2}.min^{-1}$), using exponents 0.75 ($ml.kg^{-0.75}.min^{-1}$) and 0.67 ($ml.kg^{-0.67}.min^{-1}$), and by $\dot{V}O_{2max}$ percentage.

To quantify energy expenditure we measured resting $\dot{V}O_2$ for each participant and quantified exercise energy expenditure at each velocity as multiples of this ‘individual MET’ level (Byrne et al., 2005).

Statistical analyses were performed with the Statistical Program for Social Sciences (SPSS, version 13.0). Repeated measures ANOVA followed by a *Tukey Post Hoc* test at a significance level of $p<0.05$ was used to verify intra-test differences in CHO and fat oxidation rates among speeds. Paired T-test was used to verify differences between tests in CHO and fat oxidation rates per velocity. Inter-group differences were analysed using Paired T-test.

3.5.3 Results

The preferred speed measured overground and the derived walking velocities for women (W) and girls (G), expressed as $km.h^{-1}$, respectively were: V1 = 4.5±0.6 and 3.6±0.6, V2 = 5.3±0.6 and 4.5±0.6, V3 = 6.1±0.6 and 5.3±0.6, V4 = 6.8±0.7 and 6.1±0.6. Interestingly, the same absolute velocities were seen in three situations: V1 W = V2 G, V2 W = V3 G and V3 W = V4 G.

In this study it was important that the measurement of $\dot{V}O_2$ was undertaken with participants in a steady state. All participants achieved the criterion for steady state, a difference less than 2 $ml.kg^{-1}.min^{-1}$ in $\dot{V}O_2$ between the last 2 min (Frost et al., 1995) in any 6 min exercise bout.

Values for each rating of perceived exertion scale per velocity, from V1 to V4 respectively, were for girls (OMNI scale) 1.0±0.7, 1.8±0.9, 3.1±1.2, 4.4±1.4 and for women (Borg scale) 8.4±1.2, 10.4±1.6, 12.2±1.5, 14.5±2.8.

The average intensity achieved at each velocity as a percentage of maximum heart rate for girls and women was 53±6% and 52±9%, 55±7% and 56±12%, 60±8% and 62±13%, 67±10% and 72±16%. These values represented $\dot{V}O_2$ max percentages of 31.0±8.9% and 31.0±13.2%, 34.6±10.2% and 36.3±17.4%, 40.6±11.4% and 43.4±18.5%, and 50.2±13.3% and 55.6±21.8%, respectively for girls and women (Londeree & Ames, 1976).

Results for absolute ($g.min^{-1}$), relative ($g.kg^{-1}.h^{-1}$) and proportional (%) nutrient oxidation at each relative speed and using repeated measures ANOVA are outlined in Table 3.6.

Figure 3.6 presents the relationship between energy derived from CHO and fat expressed as percentage and exercise intensity at each relative velocity for girls and women. As expected, fat oxidation reduced and CHO oxidation increased as exercise intensity increased.

Intra-group RER values were similar for girls and women with statistically significant differences between V1, V2 and V3 against V4, and between V2 and V3, for both groups.

Inter-group comparisons, again as expected, showed that age, height, body mass, body surface area (BSA), RMR and self-selected speed were significantly different ($p<0.05$). RER inter-group comparisons showed no statistical differences at any of the 4 relative velocities. Statistically significant differences were found for HR and fat (g.min^{-1}) oxidation at V1 and V2 and also CHO (g.min^{-1}) oxidation and exercise METs for all velocities. However, when CHO and fat oxidation were analysed after simple adjustment for body mass (g.kg^{-1}.h^{-1}), no difference was evident. Figure 3.7 shows Fat (g.kg^{-1}.h^{-1}) and CHO (g.kg^{-1}.h^{-1}) oxidation at each velocity for girls and women.

Figure 3.6: Percentage of CHO and FAT oxidation per velocity for girls and women

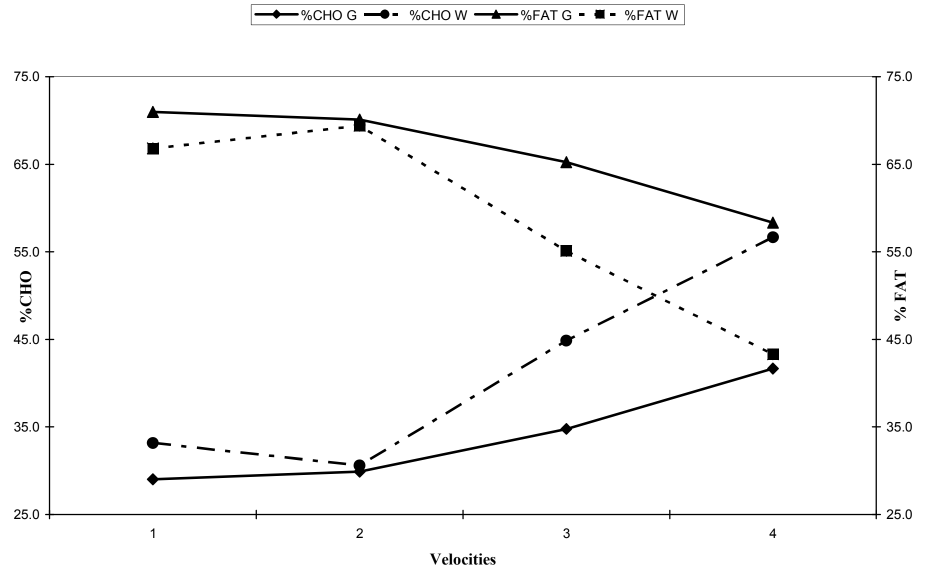
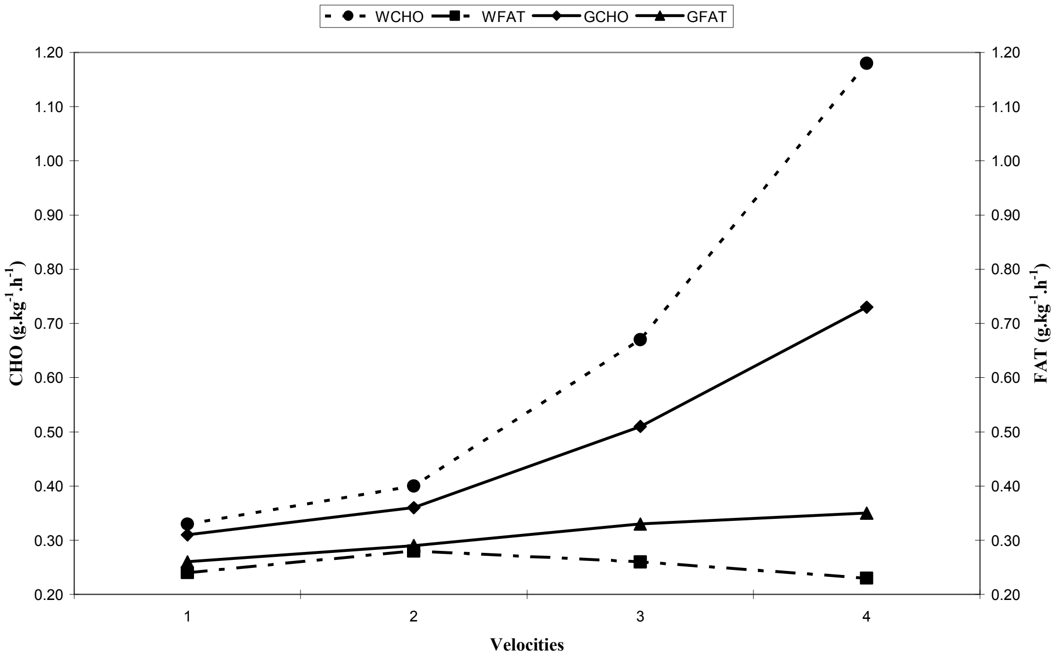


Figure 3.7: CHO and FAT oxidation per velocity for girls and women



$\dot{V}O_2$ comparisons are presented in Table 3.7. When expressing inter-group $\dot{V}O_2$ differences using absolutes values (L.min^{-1}), body surface area (ml.m^{-2}.min^{-1}) or using the exponent 0.67 (ml.kg$^{-0.67}$.min^{-1}) there were significant differences ($P < 0.05$) at all velocities. Similarly, when using an exponent of 0.75 (ml.kg$^{-0.75}$.min^{-1}) there was a significant difference at the highest velocity (V4) ($P<0.05$). However, when $\dot{V}O_2$ was expressed as a simple ratio with body mass (ml.kg^{-1}.min^{-1}) there were no significant differences.

Table 3.6 - Absolute (g.min^{-1}), relative (g.kg^{-1}.h^{-1}) and proportional (%) nutrient oxidation in each relative and absolute speed

	Girls			Women		
FAT	g.min^{-1}	g.kg^{-1}.h^{-1}	%	g.min^{-1}	g.kg^{-1}.h^{-1}	%
V1	0.14±0.03*	0.26±0.26*	71.0±10.9	0.26±0.12$^{\text{£ Ø}}$	0.24±0.13$^{\text{£}}$	66.8±23.4
V2	0.16±0.04$^{\text{*¤ Ø}}$	0.29±0.10$^{\text{*¤}}$	70.1±11.8	0.30±0.12$^{\text{£ ¶}}$	0.28±0.12$^{\text{£}}$	69.4±22.6
V3	0.18±0.04$^{\text{*¤ ¶}}$	0.33±0.10$^{\text{*¤}}$	65.2±10.9	0.28±0.16	0.26±0.16	55.1±28.4
V4	0.19±0.04$^{\text{*¤}}$	0.35±0.10$^{\text{*¤}}$	58.3±12.5	0.24±0.12	0.23±0.22	43.3±26.6
CHO	g.min^{-1}	g.kg^{-1}.h^{-1}	%	g.min^{-1}	g.kg^{-1}.h^{-1}	%
V1	0.18±0.08*	0.31±0.11*	29.0±10.9	0.35±0.27^{+}	0.33±0.27^{+}	33.2±23.4
V2	0.21±0.09$^{\text{*¤}}$	0.36±0.13$^{\text{*¤}}$	29.9±11.8	0.42±0.30$^{\text{¤}}$	0.40±0.30$^{\text{¤}}$	30.6±22.6
V3	0.29±0.14$^{\text{*¤¥}}$	0.51±0.20$^{\text{*¤¥}}$	34.8±10.9	0.71±0.49$^{\text{+¤¥ µ}}$	0.67±0.49$^{\text{+¤¥}}$	44.9±28.4
V4	0.42±0.21$^{\text{*¤¥ µ}}$	0.73±0.29$^{\text{*¤¥}}$	41.7±12.5	1.24±0.81$^{\text{+¤¥}}$	1.18±0.81$^{\text{+¤¥}}$	56.7±26.6

* Statistically significant Intra group difference ($p<0.05$) V1 against V2,V3 & V4

¤ Statistically significant Intra group difference ($p<0.05$) V2 against V3 & V4

¥ Statistically significant Intra group difference ($p<0.05$) V3 against V4

\+ Statistically significant Intra group difference ($p<0.05$) V1 against V3 & V4

£ Statistically significant Intra group difference ($p<0.05$) V1 against V2

Ø Statistically significant absolute speed Inter group difference ($p=0.02$) V1 women against V2 girls

¶ Statistically significant absolute speed Inter group difference ($p=0.008$) V2 women against V3 girls

µ Statistically significant absolute speed Inter group difference ($p=0.05$) V3 women against V4 girls

To this point, all results have been presented considering the self-selected speed and three derived velocities for both groups. However, to observe differences between girls and women during exercise at the same absolute workload we were able to compare characteristics at three velocities (4.5, 5.3 and 6.1 km.h^{-1}). Statistically significant differences for $\dot{V}O_2$ (L.min^{-1}), HR and exercise METs were verified for all measured absolute intensities. Fat (g.min^{-1}) oxidation was statistically different between women and girls for 4.5 and 5.3 km.h^{-1}, while CHO (g.min^{-1}) oxidation was only statistically different for the faster velocity (6.1 km.h^{-1}). No difference could be found for simple ratio (ml.kg^{-1}.min^{-1}), BSA (ml.m^{-2}.min^{-1}), $\dot{V}O_2$ max percentage, 0.67 exponent (ml.kg$^{-0.67}$.min^{-1}), 0.75 exponent (ml.kg$^{-0.75}$.min^{-1}), RER nor for fat and CHO (g.kg^{-1}.h^{-1}) oxidation.

Table 3.7 - $\dot{V}O_2$ responses at treadmill walking for girls and women expressed as absolute, four scaling methods and percentage

Speed	$\dot{V}O_2$ l.min^{-1}	$\dot{V}O_2$ ml.kg^{-1}.min^{-1}	$\dot{V}O_2$ ml.m^{-2}.min^{-1}	$\dot{V}O_2$ ml.kg$^{-0.75}$.min^{-1}	$\dot{V}O_2$ ml.kg$^{-0.67}$.min	$\dot{V}O_2$ max percentage
V1						
W	0.78±0.10$^{*\,Ø}$	12.4±2.8	451±71$^{¤}$	35.0±5.9	48.7±8.2$^{¥}$	30.9±5.9
G	0.43±0.10	12.7±2.8	364±74	30.5±6.4	40.4±8.3	31.0±8.9
V2						
W	0.91±0.22*¶	14.4±3.5	527±115$^{¤}$	40.9±9.5	56.9±13.1$^{¥}$	36.0±9.1
G	0.48±0.12^{Ø}	14.3±3.5	410±93	34.4±8.1	45.5±10.6	34.6±10.2
V3						
W	1.09±0.26$^{*\,\mu}$	17.4±4.2	630±139$^{¤}$	48.9±11.5	68.1±15.9$^{¥}$	43.1±10.7
G	0.58±0.15¶	17.3±4.3	496±117	41.7±10.0	55.1±13.2	40.6±11.4
V4						
W	1.42±0.37*	22.3±6.0	816±201$^{¤}$	63.3±16.3^{+}	88.2±22.7$^{¥}$	55.3±15.2
G	0.70±0.19$^{\mu}$	20.9±5.1	600±140	50.4±12.0	66.7±15.8	50.2±13.3

* Inter-group significant difference (p=0.001)

$^{¤}$ Inter-group significant difference (p=0.02)

+Inter-group significant difference (p=0.03)

$^{¥}$ Inter-group significant difference (p=0.04)

^{Ø} Inter group absolute speed significant difference (p=0.001) V1 women against V2 girls

¶ Inter group absolute speed significant difference (p=0.001) V2 women against V3 girls

$^{\mu}$ Inter group absolute speed significant difference (p=0.001) V3 women against V4 girls

3.5.4 Discussion

As expected, the absolute rates of CHO oxidation continued to rise with increased exercise intensity with the greatest utilisation during the fastest walking velocity for both groups. However for women, there were no differences in CHO utilization between the first two velocities. In contrast, a difference in rate of fat oxidation was evident for women between these speeds. This finding implies that for adult women, walking at an exercise intensity 0.8 $km.h^{-1}$ below the self-selected speed is enough to increase absolute and relative fat oxidation. At intensities above this speed, CHO oxidation predominates without an additional increase in fat utilisation (Figure 3.6 and 3.7). The crossover point occurred at the self-selected speed (Figure 3.6), but this change in oxidation rate may have been initiated at speeds only slightly faster than the previous relative velocity (self-selected speed minus 0.8 $km.h^{-1}$).

It is interesting to note that the shape of the fat oxidation curve for girls is different from that of the adult women. Fat utilisation in adults achieved a plateau at a relative velocity 0.8 $km.h^{-1}$ slower than the self-selected speed, but for girls fat utilisation increased until the self-selected speed, then stabilized until reaching the higher velocity studied. CHO oxidation curves rose abruptly above V2 for women, while for girls the acute part of this increase occurred after the self-selected speed (V3). Collectively, these results indicate that as walking intensity increases girls are able to meet the energy demands of the work by increasing fat oxidation together with the increased CHO oxidation up to the self-selected speed. In contrast, for adult women increasing CHO oxidation is associated with an early decrease in fat utilization at a velocity slower than the self-selected speed. These differences in substrate utilization between prepubertal girls and women are in agreement with others (Martinez & Haymes, 1992) suggesting that girls rely more on fat and less on CHO utilization than women during treadmill walking at relative intensities based on self-selected velocities.

In agreement with our results in girls and women, a study in which substrate utilization of pre- and post-pubertal boys was compared showed that post-pubertal boys had an earlier shift from a greater dependence on fats at low intensities towards a preferential use of CHO during exercise than the pre-pubertal boys (Brandou et al., 2006). The shift in substrate selection with increased exercise intensity is under multiple regulatory controls. However, physiologic characteristics inherent to aerobic metabolism in children could, at least partially, explain why children have an enhanced ability to generate energy from oxidation of fat in exercising muscles due to higher activity of aerobic enzymes in skeletal muscle (Berg, Kim, & Keul, 1986), a higher proportion of type I fibres in the vastus lateralis muscle compared to untrained adults (Eriksson & Saltin, 1974; Bell, MacDougall, Billeter, & Howald, 1980), slightly greater mitochondrial volume, ratio of mitochondria to myofibrillar volume and intramuscular lipid storage (Bell et al., 1980).

The accuracy of indirect calorimetry measurement during exercise with increased CO_2 production (above the anaerobic threshold), and further increases in RER independent of the balance of substrates has been discussed (Kanaley, Mottram, Scanlon, & Jensen, 1995; Perez-Martin, Dumortier, Raynaud, Brun, Fedou, Bringer, & Mercier, 2001). In the present study during the higher walking intensity the heart rate percentages achieved for girls were 67% which are relative to ~ 50% $\dot{V}O_2$ max. On the other hand, for women walking at the fastest speed, %HR max was 72% or representative of ~ 56% of $\dot{V}O_2$ max. It may be argued that this intensity would be high enough to reach the ventilatory anaerobic threshold in an untrained population, which typically occurs at around 55-60% $\dot{V}O_2$max introducing bias in our comparisons because the ventilatory anaerobic threshold of children reportedly occurs at a higher percentage of $\dot{V}O_2$ max than in adults (Nixon, 2000). However, comparisons between indirect calorimetry and more stringent techniques have showed that absolute substrate oxidation could be determined independently of a stable bicarbonate pool (Romijn et al., 1992).

It must be noted that the absolute rates of fat oxidation are dependent on CHO intake (Achten et al., 2002) consequently affecting the contribution of CHO to total energy expenditure. To prevent this influence on oxidation rates, all exercise tests in the current study were performed in a fasting state of at least 12 hours.

When comparing groups of individuals of different body mass, such as girls and women in the present study, the two most common theoretical scaling exponents in the literature are 0.67 from the theory of geometric similarity where human power output is proportional to stature^2 or body mass$^{0.67}$(Astrand, 1976) and 0.75 which is based on comparative zoology studies of mass$^{0.75}$ (Kleiber, 1947). However, there is reference in the literature to mass exponents for peak $\dot{V}O_2$ in females ranging from 0.23 to 0.97 (Rowland, 1997b; Welsman, 1997).

No scaling exponent used in this study (Table 3.7) was able to eliminate $\dot{V}O_2$ differences verified in absolute values between girls and women. An exception could be seen when using a simple ratio with body mass. Maximal $\dot{V}O_2$ is not only affected by body size but by numerous other factors such as motivation, fitness level, training and genetics and despite the fact that these factors might also affect submaximal $\dot{V}O_2$, their influence would be expected to be considerably less (Rogers et al., 1995) because submaximal $\dot{V}O_2$ does not increase proportionally to body mass (Bergh, Sjodin, Forsberg, & Svedenhag, 1991). Submaximal mass exponents of 0.89, 0.90 and 0.93 for velocities of 8, 9 and 10 km.h^{-1}, respectively, demonstrated how a theoretically predicted exponent for peak $\dot{V}O_2$ does not enable the same exponent to be used to control for body mass differences in $\dot{V}O_2$ during submaximal exercise (Armstrong, Welsman, & Kirby, 1999). A previous study comparing submaximal $\dot{V}O_2$ between children and adults using analysis of covariance to control for body mass indicated that there was no real difference in the dynamics of $\dot{V}O_2$ for men and boys and indicated that any apparent difference in $\dot{V}O_2$ between groups may be attributable to inappropriate analysis rather than inherent physiological differences (Eston, Robson, & Winter, 1993).

Differences verified in absolute $\dot{V}O_2$ values were normalized using a simple ratio with body mass. We were unable to determine our own exponents because the greatest variation in exponent determination occurs when using small samples sizes (Rogers et al., 1995). However, body mass exponents of 0.95 (Ross et al, 1991) and 1.01 (Sjodin & Svedenhag, 1992) have been reported in the literature and a wide range of exponents ranging from 0.60 (Bergh at al., 1991) to 1.01 (Sjodin & Svedenhag, 1992). Given this large variability in scaling values it is not surprising that our values were normalised using a simple ratio with body mass. Our results are supported by others (Krahenbuhl, Skinner, & Kohrt, 1985; Rowland & Green, 1988) who also consider that simple ratio scaling is an appropriate method of accounting for growth-related changes since total body mass is lifted, lowered and supported during walking tasks. Although numerous studies have presented substantive arguments against the use of the simple ratio standard of body weight, a clear alternative has not surfaced (Rogers et al., 1995).

As nearly all locomotor activities are performed at less than maximal intensity, a useful index of the energy expenditure associated with movement is economy of locomotion, defined as the oxygen consumption for a given submaximal speed or power output (Morgan, 2000). In both groups SS was the most economical speed considering the amount of energy consumed per unit distance expressed as $\dot{V}O_2$ gross [$\dot{V}O_2$ (ml.kg^{-1}.min^{-1}) divided by speed (m.min^{-1})]. Serendipitously, we were also able to compare three absolute velocities between groups. Our results are in agreement with the literature (Rowland & Green, 1988; Martinez & Haymes, 1992; Armstrong, Kirby, Welsman, & McMannus, 1997b) showing that girls are less economical than women at all the speeds assessed. However, from a substrate utilization perspective, the absolute work comparisons only showed a statistically significant difference for absolute CHO oxidation (g.min^{-1}) at the highest speed with women displaying higher CHO utilisation. Fat oxidation (g.min^{-1}) differences were seen at the two slower speeds with higher fat oxidation for girls. Again, as in the analyses based on the self-selected speed, when CHO and fat oxidation were analyzed after simple adjustment for body mass (g.kg^{-1}.h^{-1}), no difference could be found. However, walking comparisons at absolute velocities between participants of different maturational status would involve a wide range of exercise intensities and be affected by variability in gait efficiency resulting in limited interpretations on substrate oxidation. Our strategy to use derived velocities based on the self-selected walking speed was chosen to provide a more appropriate basis for comparisons. We wanted exercise intensities to be relative to perceived exertion and to use a reference workload that was applicable in free-living conditions.

It is also interesting to compare the females in different age groups in terms of the individualised exercise METs. The origin of the value of 3.5 $ml.kg^{-1}.min^{-1}$ equivalent to 1 MET is vague. This value is commonly accepted for adults and is reported in most scientific textbooks; however recently there has been some critical debate in the literature regarding the use of this standard figure for all people irrespective of size. We have previously shown that this standard value overestimates the actual resting $\dot{V}O_2$ value on average by 35%, and the 1-MET of 1 $kcal.h^{-1}$ overestimates resting energy expenditure by 20% (Byrne, Hills, Hunter, Weinsier, & Schutz, 2005). Further, we have reported in children that this standard value underestimates the resting $\dot{V}O_2$ value on average by 25%, and resting energy expenditure by 30% (Amorim, Byrne, & Hills, 2006). In the present study we have used the individualised MET value; with exercise $\dot{V}O_2$ expressed as a multiple of resting $\dot{V}O_2$. As expected, resting $\dot{V}O_2$ values in children were higher than seen in women. When using individual MET values to quantify exercise energy expenditure, exercise METs were still significantly different between the age groups with women showing higher values at all absolute and relative velocities. This result could be useful when utilizing thresholds to delineate intensity of physical activities for girls and women, and also for predicting the energy costs of physical activity prescriptions. Regarding energy expenditure of physical activity, the use of individual MET values could be an interesting and applicable tool to compare children and adults, independent of the complexity and lack of a universally accepted alternative of body size scaling.

The most important finding of this study was the different relationship between girls and women in the proportional energy derived from fat and CHO during treadmill walking at velocities based on self-selected speed. In adult women fat utilization starts to decrease at a velocity slower than the self-selected speed, whereas for girls the fat oxidation rate starts to plateau at this velocity. Our findings for women are consistent with the “crossover concept” of fuel utilization around 50% of $\dot{V}O_2$ max (Brooks & Mercier, 1994) and in agreement with a recent study conducted in a large sample showing the crossover point between 48 and 53% of $\dot{V}O_2$ max (Venables et al., 2005). For girls the speeds utilized were not intense enough to verify the crossover point, probably due to the delayed anaerobic energy contribution occasioned by lower rates of the phosphofructokinase enzymes limiting the availability for glycolysis in children (Eriksoon and Saltin, 1974) and the verified enhanced fat utilization.

Morgan (2000) showed that walking at a freely chosen speed required the least amount of energy compared to the curvilinear rise in $\dot{V}O_2$ observed as speed varied away from the preferred condition. We showed in this study that the fat oxidation reduction and the CHO utilization increase was close to the self-selected speed for girls and women, a velocity easily achieved in daily activities. As a strategy for increased exercise adherence and to avoid fatigue without lost of exercise efficiency on substrate utilization, plus avoid the reduced gait efficiency habitually shown at increased walking speeds, we propose the use of the self-selected speed as an upper cut-off point for intensity control during exercise for both age groups.

Chapter IV – ENERGY EXPENDITURE IN CHILDREN DURING FREE-LIVING ACTIVITIES

4.1 Literature Review

Measures of children's energy expenditure

The most widely used laboratory technique to estimate energy expenditure is indirect calorimetry, which estimates expenditure based on oxygen consumption. Despite the limited number worldwide, whole body calorimetry is the laboratory technique of choice (Webb, Saris, Schoffelen, Van Ingen Schenau, & Ten Hoor, 1988; Ballor, Burke, Knudson, Olson, & Montoye, 1989; Durnin, 1996; Montoye, Kemper, Saris, & Washburn, 1996), whereas the field technique of choice is doubly labelled water (DLW). The development of the DLW technique can be traced to a study performed by Lifson et al. in the 1940s (Schoeller, Bandini, Levitsky, & Dietz, 1988). The technique is non-invasive and non-restrictive and thus is ideal for the measurement of total daily energy expenditure in free-living subjects (Schoeller et al., 1988; Melanson & Freedson, 1996; Schoeller, 1999).

Other commonly employed field techniques include self-report questionnaires, estimates of energy expenditure based on physiological response to activity (for example, heart rate), direct observation, factorial methods, and movement sensors (such as pedometers and accelerometers) (Gretebeck & Montoye, 1992; Haskell, Yee, Evans, & Irby, 1993; Boulay, Serresse, Almeras, & Tremblay, 1994; Bouten, Westerterp, Verduin, & Janssen, 1994; Bray, Wong, Morrow, Butte, & Pivarnik, 1994; Fogelholm, Hiilloskorpi, Laukkanen, Oja, Van Marken Lichtenbelt, & Westerterp, 1998; Weller & Corey, 1998). Many of these techniques have limitations, including that they have not been validated for a wide range of populations across both genders and a range of ages, and in addition many lack precision (Melanson and Freedson, 1996; Armstrong, 1998).

Direct observation is an appropriate criterion measure of physical activity, however the technique is impractical. Studies have compared direct observation scores with heart rate or oxygen consumption and reported correlations ranging from r = 0.61 to 0.91 (Epstein, McGowan, & Wooddall, 1984; O'Hara, Baranowski, Simons-Morton, Wilson, & Parcel, 1989) with satisfactory inter-observer agreement (84 to 99%) among simultaneous observations of the same child (Puhl, Greaves, Hoyt, & Baranowski, 1990; McKenzie et al., 1991) . On the other hand, Telford et al. (2004) found poor validity of both the proxy (r = -0.04 to 0.90) and the self-report (r = -0.04) instrument in estimating physical activity participation in individuals and suggested that children cannot accurately report physical activity duration as well as their parents. Specific drawbacks of direct observation include the relatively high experimenter burden and the potential reactivity of the study participants (Sirard and Pate, 2001).

The factorial method estimates energy expenditure using tabulated data for task-specific metabolic costs, the participant's basal metabolic rate, and the amount of time spent on a task. This method has been widely used but has a number of problems when used with children. Most importantly, the task-specific tables are primarily applicable to adults (Ainsworth, Haskell, Leon, Jacobs, Montoye, Sallis, & Paffenbarger, 1993; Ainsworth et al., 2000), despite some recent efforts to provide references to children's activity (Harrell et al., 2005).

Doubly labelled water technique – The gold standard

Schoeller and van Santen (1982) documented the first applications of a stable isotope technique that had the potential for accurate and objective measurement of TEE in free-living individuals. This approach, known as the doubly labelled water technique (DLW), has been adopted by many laboratories throughout the world, and applied, in a wide range of diverse circumstances. The DLW technique has several advantages for evaluating energy expenditure as it can be easily used in free-living (normal daily life) and has low reactivity. The technique is currently the most objective method to assess energy expenditure (Schoeller, 1999). The DLW technique is accurate and has a precision between 2 and 8%, depending on the dose, the length of the metabolic period measured, and the number of samples (Schoeller, 1988) and has repeatability between 6 and 10% (Schoeller & Hnilicka, 1996).

Over the past decade many studies have used the DLW technique in studies on children. For example, (Hoos et al., 2003a) completed a meta-analysis that considered data from 17 studies in children of both genders, aged between 3 and 16 years. Low values found in young children increased to adult values with age, although children may appear to be more active than adults. Although children need more energy per kilogram body weight to perform a particular activity than adults (Waters et al. 1988), their lower body weight leads to smaller overall energy expenditure compared to older subjects.

The DLW technique has been used for validation studies against other tools including physical activity records (Bratteby, Sandhagen, Fan, & Samuelson, 1997; Koebnick et al., 2005), dietary intake (Black & Cole, 2000; McGloin et al., 2002), motion sensors (Bouten, Verboeket-van de Venne, Westerterp, Verduin, & Janssen, 1996; Ekelund et al., 2004b; Reilly, Kelly, Montgomery, Jackson, Slater, Grant, & Paton, 2006), dietary intake (Trabulsi, Troiano, Subar, Sharbaugh, Kipnis, Schatzkin, & Schoeller, 2003; Montgomery, Reilly, Jackson, Kelly, Slater, Paton, & Grant, 2005) and heart rate (Livingstone et al., 1992; Davidson et al., 1997). Indirect estimation using the stable isotope technique has the disadvantage that only total daily energy expenditure over several days can be estimated, not energy expenditure for any specific activity (Livingstone et al., 1992).

In summary, it is important to note that the DLW technique does not provide specific information about activity patterns and energy expenditure associated with physical activity (Lazzer, Boirie, Bitar, Montaurier, Vernet, Meyer, & Vermorel, 2003). Total energy expended in physical activity is an important variable but it is necessary to consider other dimensions of activity such as frequency, intensity and duration, rather than simply the global measure of energy expenditure possible using DLW. For this purpose, investigators should use accelerometers, heart rate monitors, or a combination of the two (Bassett, 2000).

Indirect and objective measurement of energy expenditure - Accelerometers

Accelerometers are useful because they are very small and relatively low-cost instruments that provide quantitative measurements. These devices allow measurement of accelerations and decelerations caused by movement of the body and have the potential to reflect the intensity of movement (Westertep, 1999), as well as postural orientations (Mathie, Celler, Lovell, & Coster, 2004a).

The acceleration of a point on the body, measured with an accelerometer, is directly proportional to muscular force and therefore related to energy expenditure (Benefice & Cames, 1999). For activities that have dynamic properties, the direct relationship between body acceleration and energy expenditure can be used, but an important limitation of accelerometry is that it does not measure static components of exercise. However, in normal daily life, it is assumed that the effect of static exercise on the total level of physical activity is negligible (Westertep, 1999).

Many accelerometers only measure movement in one axis and therefore appear to have limited value for the assessment of energy expenditure in field studies in which activities involve multi-directional components (Johnson, Russ, & Goran, 1998; Allor & Pivarnik, 2001). Accelerometers utilise movement counts or ‘vector magnitude’ to subsequently calculate energy expenditure for each minute, however the equations used to convert accelerometer outputs to energy expenditure from physical activity are proprietary and therefore not released by the manufacturer. Many researchers have referenced the difficulty of accepting the presentation of results which are based on ‘mysterious’ calculations that cannot be readily discussed (Rodriguez et al., 2002).

There are clear limitations regarding what can be measured in a free-living monitoring environment using a single, waist-mounted, triaxial accelerometer. The use of a number of objective instruments may provide more information that allows for more accurate classifications, however a single instrument is more practical for continuous, long-term monitoring, as the simplicity and ease-of-use of the single instrument facilitates compliance and minimises cost (Mathie, Coster, Lovell, & Celler, 2004b).

An accelerometer positioned at the waist is generally chosen because this location provides the most useful information on a participant’s movements, being close to the centre of the body and reported as being comfortable (Mathie et al., 2004a). Other approaches, such as wristbands and pendants, require less participant compliance as they need never be removed; but they are less able to provide reliable information on whole body movements and are more susceptible to artefact, such as knocking against other objects (Mathie et al., 2004b).

Activity monitors have been validated against indirect calorimetry and calibrated in terms of resting metabolism equivalents (METs). The MET thresholds used to quantify the time spent in light (<3 METs), moderate (3 to 6 METs), hard (6 to 9 METs), and very hard (>9 METs) activities have only been defined in adults (Freedson, Melanson, & Sirard, 1998). The usual practice of defining 1 MET as 3.5 mL O_2/kg per minute is incorrect in children, because their resting metabolic rate declines from ~ 6 mL O_2/kg per minute at 5 years of age to 3.5 mL O_2/kg per minute at 18 years of age (Schofield, 1985).

Activity monitors have been calibrated to detect moderate to high physical activity levels, and therefore do not discriminate sedentary activities such as watching television and playing video and computers games. The accuracy of the time spent in sedentary activities when reliant on participant recall is low and could be improved with the use of activity monitors calibrated with a sedentary threshold (Dietz, 1996).

Freedson et al. (2005) and Trost et al. (1998) have developed age-specific accelerometer count ranges for children. However, adult-derived thresholds are not applicable to children and can lead to erroneous conclusions regarding physical activity levels in this population (Puyau et al., 2002). Puyau et al. (2002, 2004) defined threshold levels for children using accelerometers, not in terms of METs, but in terms of the energy expended in activity (AEE = EE - BMR), or physical activity ratio (PAR = EE/BMR). They found that the relationship between AEE and activity counts was independent of age and sex. These authors concluded that activity monitors can be used to assess not only moderate to vigorous physical activity, but also physical inactivity, as they were able to distinguish sedentary from light activities.

Anderson et al. (2005) evaluated the validity of the Previous Day Physical Activity Recall (PDPAR) using accelerometry as the criterion measure and compared estimates of time spent in moderate and vigorous physical activity with Freedson et al. (1997), Trost et al. (2002) and Puyau et al’s. (2002) age-adjusted cut-point methods. This study showed that the diary estimates for vigorous activity were substantially higher than all cut-point methods used, suggesting a systematic misclassification of moderate activity as vigorous. One important factor attributed by the author regarding the inability of the accelerometer to detect many types of vigorous physical activities was the sampling interval of 1 minute (epoch time). The activity patterns of children and younger adolescents are characterised by shorter, more explosive bouts of activity (Bailey et al., 1995). Unpublished studies by Sjostrom et al. and cited by Anderson et al. (2005) have shown that when using a shorter epoch time such as 30 seconds, the number of minutes spent in vigorous activity increased significantly. The advent of high-frequency accelerometers has allowed measurement using epochs as short as 1 second. However, we can only identify two studies using short epochs to verify children's physical activities in free-living conditions. Ridgers et al. (2005) used a 5 second epoch to assess physical activity pattern in children during recess at school, the limitation being that there was only one day of monitoring. Recently, Baquet et al. (2007) examined the duration of physical activity bouts in children under free-living living conditions across 7 days using an epoch setting of 2 seconds. Both studies showed significant discrepancy between the times recorded with different epochs, emphasising the need for the use of a short epoch interval to determine children's real physical activity patterns.

Ekelund et al. (2001) evaluated the validity of accelerometry to assess the amount of physical activity associated with energy expenditure estimates from DLW and found that the method was a useful tool. Reilly et al. (2004) measured TEE by DLW and physical activity by accelerometry in young children and found that children typically spent only 20-25 min per day in moderate-to-vigorous physical activity. Abbott and Davies (2004), in an interesting approach using DLW and accelerometry, found that minutes spent in either vigorous or hard activity were not correlated with overall TDEE or PAL, suggesting that these activity parameters do not represent the same dimension of activity. In this study, data was collected over a specified 10-day period for energy expenditure and 4 days for accelerometry. The authors recognised that an analysis of 7 days might have been a better reflection of patterns of intensity.

Trost et al. (2000) examined age-related differences in the reliability of objectively measured physical activity and indicated that between 4 and 5 days of monitoring would be necessary to achieve a reliability of 0.80 in children (grades 1 to 6), and between 8 to 9 days of monitoring to achieve the same reliability values in adolescents. For all grade levels, between-day intraclass reliability coefficients for a 7-day monitoring period were within an acceptable range (R = 0.76-0.87).

An interesting study conducted by O'Connor et al. (2003) considered a two-hour after-school period measured using a three-day parent-reported activity diary, a parent-reported physical activity questionnaire, the Tritac-R3D accelerometer and DLW. This study reported a high correlation between activity indices from the parent-reported diary and the Tritac which indicated that a specific, easy to record, two-hour after-school period was representative of a child's average activity level. Mota et al. (2003) examined the weekday patterns of moderate-to- vigorous physical activity (MVPA) using accelerometers and verified that boys were engaged in more MVPA than girls in general, but girls tended to be more active during the school period, while boys were more active after school. Results of both previously cited studies were in agreement with the suggestion of Welk et al. (2000) that the time after school may be a critical period that defines a child's physical activity level.

Schutz et al. (2001) proposed the Activity-Related Time Equivalent Heart Rate (ARTE*HR*) as a potentially useful tool as well as an index using counts by accelerometry as a useful extrapolation of Activity-Related Time Equivalent accelerometry (ARTEaccel). This approach was used later by Ekelund et al. (2003) in adolescents. Rowland et al. (2004) used an RT3 accelerometer and proposed count thresholds relating to moderate and vigorous intensity activity.

The position of attachment of an accelerometer to the body can also influence accelerometer output. Ideally, several accelerometers are attached to different sites on the body; however this may be very unpleasant for some participants. Accelerometers are commonly attached to the waist while others are attached to the hip (Hoos, Plasqui, Gerver, & Westerterp, 2003b).

Accelerometers have been used to measure arm movements and serve as an ergonomic tool to assess risk factors for upper extremity work-related disorders (Estill, MacDonald, Wenzl, & Petersen, 2000). Further, they have been used as an inclinometer for field measurements of arm postures and movements (Bernmark & Wiktorin, 2002), and as an objective measure for patients' rehabilitation with upper-extremity impairments (Uswatte, Miltner, Foo, Varma, Moran, & Taub, 2000; Vega-Gonzalez & Granat, 2005). However, Leenders et al. (2003) showed that prediction of energy expenditure with measurement of bodily movement at the hip explained 74% of the variance in energy expenditure and accelerometers worn at the wrist showed low predictability. Swartz et al. (2000) in a field experiment showed that the output from an accelerometer worn at the wrist explained only 3% of the variation in energy expenditure and combined with the hip output, the information added little improvement in predicting energy expenditure.

Equations based purely on locomotor activities tend to underestimate TEE or physical activities because such equations or cut-off points are based on activities that have a relatively high movement-to-EE-ratio. Lifestyle activities involving a significant amount of upper body movement tend to have a higher energy cost for the amount of movement detected, and this increased energy cost would not be captured by these equations (Welk, McClain, Eisenmann, & Wickel, 2007).

Accelerometers have also been shown to overestimate the energy cost of sedentary activities (Strath, Bassett, & Swartz, 2003) and underestimate the energy cost of high intensity activities (Meijer, Westerterp, Koper, & ten Hoor, 1989). However, Rowlands et al. (2004) made an interesting observation and suggested that sedentary and light activities be used in accelerometer validation studies because the majority of the waking day is spent in sedentary activities in both children and adults.

Physical activity levels and patterns of 2185 European children measured using the accelerometry technique showed this measurement approach to be both feasible and accurate for use in large epidemiological studies of children's activity (Riddoch et al., 2004). These small, unobtrusive devices have the capacity to store movement data for periods of time as long as 22 days, depending on the epoch length. Therefore, as a means of gaining objective information regarding frequency, intensity, and duration of activity, accelerometers are good tools to use in studies regarding patterns of physical activity.

Indirect and objective measurement of energy expenditure - Heart rate methods

The well-known relationship between energy expenditure and heart rate has led to the use of heart rate monitoring as a method for estimating energy expenditure (Spurr, Prentice, Murgatroyd, Goldberg, Reina, & Christman, 1988a; Wareham, Hennings, Prentice, & Day, 1997). Under controlled laboratory conditions during dynamic exercise, heart rate and $\dot{V}O_2$ are closely related and exhibit a linear relationship (Freedson and Miller, 2000). Although variable between people, within individuals HR and oxygen uptake tend to be linearly related throughout a wide range of aerobic exercise tasks (Livingstone, 1997). When this relationship is known, the exercise HR can be used to estimate $\dot{V}O_2$ and then compute energy expenditure during free-living conditions. This principle is not new and stems from the work of Berggren and Christensen (1950), and some of the most promising current developments have been using this methodology (Livingstone, 1997; Strath, 2000; Iannotti et al., 2004).

Amongst the various physiological variables used to estimate energy expenditure, heart rate has widespread applicability (Montoye et al., 1996; Freedson & Miller, 2000). An appreciation of relative merits of the method is particularly important when studying children because most of available field techniques are likely to induce behavioural changes in children’s spontaneous and natural activity patterns (Livingstone et al., 2000). Since the late 1970s, the development of portable heart rate devices has broadened the potential usefulness of this relatively low-cost technique for quantifying daily energy expenditure in ‘real world’ situations (Leonard, 2003).

One of the first attempts to predict energy expenditure from heart rate was based on accumulated or average heart rate over the entire period of time under investigation (Bradfield, Chan, Bradfield, & Payne, 1971; Payne, Wheeler, & Salvosa, 1971). However, this approach received several critiques (Dauncey & James, 1979; Christensen, Frey, Foenstelien, Aadland, & Refsum, 1983), mainly because the average heart rate over 24 hours does not rise very much over values found in resting conditions where the $\dot{V}O_2$-HR relationship is least precise (Spurr et al., 1988). The development of portable heart rate devices able to measure and store heart rate minute-by minute was a significant advancement over the older heart rate accumulation method.

A common practice since early studies has been to determine for each participant, an individually-calibrated curve to establish the $\dot{V}O_2$-HR relationship, and then use the curve to convert heart rate recorded under field conditions into oxygen uptake and subsequently into energy expenditure. A major shortcoming of this method, however, is the uncertain relationship between $\dot{V}O_2$-HR in sedentary activity despite a good linear relationship at levels of physical activity above basal and below maximal output (Dauncey and James, 1979; Livingstone et al., 1992).

Allor and Pivarnik (2001) examined the stability and convergent validity of HR and verified that intra-class reliability coefficients between 2 days of HR monitoring were 0.99 and single-day reliability coefficients were 0.98. These results showed that HR was extremely stable in girls during normal schools days.

Spurr et al. (1988a) developed a method named 'Flex heart rate' (flex-HR), an individually-predetermined HR that can be used to discriminate between resting and exercise HR. The flex-HR method was validated in adults against whole-body calorimetry (Spurr et al., 1988a; Ceesay, Prentice, Day, Murgatroyd, Goldberg, Scott, & Spurr, 1989; Bitar, Vermorel, Fellmann, Bedu, Chamoux, & Coudert, 1996) and also DLW (Livingstone et al., 1990) with good agreement for group comparisons. Livingstone et al. (1992) using DLW and HR showed that flex-HR was a low-interference technique for accurately predicting group estimates of habitual total energy expenditure in healthy, free-living children.

The flex-HR method has been applied to verify the minutes spent in different intensities by Ekelund et al. (2000) to assess TDEE and patterns of physical activity in adolescents and to relate the amount and intensity of physical activity to existing recommendations. Similarly, Grund et al. (2000) used the approach to verify the effect of gender on different components of TDEE in free-living pre-pubertal children. An important consideration of the latter study was that gender had no significant effect on flex-HR.

The problem with the use of two separates lines to describe the relationship between EE and HR is that the accuracy of the final results depends on the appropriateness of an individually predetermined HR that can be used to discriminate between resting and exercise HR (Li, Deurenberg, & Hautvast, 1993). The authors suggest that the relationship between EE and HR differs between individuals and within individuals on different occasions, therefore it is necessary to develop individual calibration curves immediately before the HR recording period. Other studies have confirmed this suggestion (Panter-Brick, Todd, Baker, & Worthman, 1996; Spurr, Dufour, Reina, & Haught, 1997; Murayama & Ohtsuka, 1999) supporting the generalisation that the flex-HR method of estimating EE is reliable at the population level, but not necessarily stable for individuals over time. One important consideration is that the best value of the flex-HR may be participant-specific but may also depend on the mode of activity used to determine the $\dot{V}O_2$-HR relationship which differs among investigators (McCrory, Mole, Nommsen-Rivers, & Dewey, 1997). The within-participant differences found when comparing heart rate monitoring with whole-body calorimetry (Ceesay et al., 1989) and DLW (Livingstone et al., 1992), have been attributed mostly to the low correlation between HR and EE during sedentary activities.

McCrory et al. (1997) examined the between-day and within-day variability in the $\dot{V}O_2$-HR relationship and the effect of this variability on the estimation of TEE. The investigators concluded that the minute-by minute HR method is appropriate for estimation of TEE in groups due to the excellent average between-day and within-day repeatability of the $\dot{V}O_2$-HR relationship.

The individual nature of the HR-$\dot{V}O_2$ relationship makes it necessary to establish a regression equation for HR-$\dot{V}O_2$ for each individual at several levels and intensities of activity while recognising that factors other than $\dot{V}O_2$ consumption can influence HR. These factors include ambient temperature, emotions, food intake, body position, the muscle group exercised, whether the exercise is continuous or stop-and-start, or whether the muscles are contracting isometrically or in a more rhythmic manner (Livingstone, 1997; Achten & Jeukendrup, 2003). It is necessary to consider that HR also tends to return to resting levels more slowly than oxygen uptake following physical activity (Spurr et al., 1988). Provided that the limitations of the method are recognised and errors in estimating TEE can be tolerated (Livingstone, 1997), its accuracy could be improved by repeated measurements of HR and EE at rest and light activities, various types of usual activities and exercise, and measurements of HR and EE during the recovery periods of exercise. This method could then be accurate enough to predict TEE of individuals (Bitar et al., 1996). Treuth et al. (1998) supports this contention using a combination of HR and activity monitoring.

The flex-HR method has contributed substantially to our understanding of variability in human energy expenditure and ongoing research, including examination of variability in the flex-point and the incorporation of activity monitors into the protocol, are helping to improve the accuracy and expand the utility of the technique (Leonard, 2003).

The identification of a reproducible flex-HR to distinguish between resting and activity HR also remains problematic since it is based on the tenuous assumption that one discrete pulse point can provide a clear-cut distinction between rest and exercise (Livingstone et al., 2000). Recently, Iannotti et al. (2004) found that individualised equations using the full range of laboratory HR values, and two equations, one for HR in the range of non-treadmill activities measured and one for HR above this range, were significantly better than the flex-HR method in estimating EE within the laboratory protocol. However, group calibration curves may increase the error of estimating EE (Rowlands, Eston, & Ingledew, 1997).

Efforts to improve the accuracy of TEE from HR estimates should be ongoing. The benefits of any additional complexity in the methodology should not detract from the fact that HR monitoring remains one of the most feasible and cost-effective techniques for assessing TEE and/or associated patterns of physical activity under difficult and remote field conditions. Under such circumstances, the selection of calibration activities will inevitably be dictated by the facilities available for measuring respiratory gas exchange and the homogeneity or heterogeneity of habitual physical activity patterns (Livingstone et al., 2000).

Simultaneous use of heart rate and accelerometry for the measurement of energy expenditure

The study by Avons et al. (1988) was the first to examine the value of combining heart rate and movement sensing to increase energy expenditure measurement precision. The conceptual basis and preliminary evaluation of a procedure using the simultaneous recording of heart rate and two motion sensors was applied by Haskell et al. (1993). This early paper described an important concern about HR monitoring using the motion sensor data to determine when the person was active and establish if the activity was primarily leg exercise, arm exercise or a combination of the two. This procedure does not include increased EE estimated from an elevation in HR due to non-exercise reasons and provides a more accurate estimate of EE than possible using a regression determined for leg exercise.

Moon and Butte (1996), using a room calorimeter, identified active and inactive periods of physical activity (PA) by counts from a vibration sensor and HR threshold. The sleep (inactive) and awake (inactive) periods were determined by inspecting HR and PA records for a rapid decline in HR followed by >1 h of minimal HR and PA close to zero. The intersection of the inactive and active equations for $\dot{V}O_2$ showed that this method achieved improved precision in $\dot{V}O_2$ estimates by the classification of 'awake' HR to active and inactive functions using PA.

During both low-intensity activity and very high intensity activity, heart rate fails to maintain a linear relationship with oxygen consumption or EE. The vast majority of the physical activity undertaken by an 'average' individual is low intensity throughout the day. The addition of one or more motion sensors to heart rate monitoring for the measurement of daily energy expenditure appears to be warranted (Luke et al., 1997) and enable a better prediction of oxygen consumption in a laboratory setting that simulated activities of daily life than HR alone. However, the addition of motion sensor information to HR did not improve the prediction of $\dot{V}O_2$ for the higher intensity exercise during a submaximal treadmill test.

Eston et al. (1998) reported a higher correlation of scaling $\dot{V}O_2$ (s$\dot{V}O_2$: body mass raised to the power of 0.75) with the triaxial movement measure than with the HR measure during selected children's activities. When the two measures were used simultaneously this provided the best estimate, as has been detailed in the adult literature (Haskell et al., 1993; Luke, Maki, Barkey, Cooper, & McGee, 1997). However, these authors considered that the increase in known variance attributable to HR would not justify the additional cost and labour. A limitation of the Eston study was the reliance on raw HR data to predict s$\dot{V}O_2$.

Applying the combined HR/activity method Moon and Butte (1996) verified that the combination of HR and activity is an acceptable method for determining EE for groups of children and also for individuals. In this study a sensor was taped to the leg to measure activity, however there may have been several types of activities that elevated HR but did not involve leg movement. The authors suggested that it might be worthwhile to add another sensor to the arm to capture all movement and potentially improve estimates of $\dot{V}O_2$ and EE.

Using a prototype of a new device capable of recording HR and body movement simultaneously, Rennie et al. (2000) showed a more precise estimate of group EE. The standard deviation of the mean percentage error was smaller than the flex-HR method compared with a whole-body calorimeter. If the use of a movement sensor in combination with HR can reduce the overall error of the two instruments, such a device could be a good tool to estimate PAEE in population-based studies. This is consistent with the work of Freedson and Miller (2000) who recommended the development of an objective and simultaneous HR and motion analysis system. In short, it is important to verify that elevated HR represents a physical activity response.

Strath et al. (2001) using simultaneous HR-motion sensor and a portable metabolic measurement system, performed individual HR-$\dot{V}O_2$ calibration curves for both arm and leg activities, using the approach suggested by Treuth et al. (1998). Strath et al. (2001) did not examine combined arm and leg activity as this closely reflected the legs-only condition by Haskell et al. (1993). Alternatively, a ratio between arm and leg counts was used. A ratio of greater than or equal to 25 reflected arm work, whereas a ratio of less than 25 represented leg work and this determined which regression equation was used. The simultaneous HR-motion sensor technique showed a significantly higher relationship with $\dot{V}O_2$ for all participants than HR alone ($P<0.001$) and the hip-mounted motion sensor ($P<0.001$). The same group of researchers (Strath et al., 2002) using the same approach as in their first study (Strath et al., 2001) found that the HR-motion sensor technique had a greater level of agreement than the flex-HR method for the amount of time spent in all activity categories in comparison with indirect calorimetry.

Plasqui and Westerterp (2005) utilised a new approach with accelerometry-HR as a measure of physical fitness and suggested that after correction for body composition their proposed index and $\dot{V}O_2$ max was significantly related. This was achieved without the need for a specific protocol or for maximal exertion, but rather by monitoring people in their daily life activities.

The precision of any objective assessment method, especially EE estimated from HR, is dependent on some level of individual calibration. However, this procedure places additional demands on both experimenter and participant. Brage et al. (2004) using the combination of HR and motion-sensor data in a branched equation model verified improvements in estimates of PAEE in a population of trained young men compared with either method used alone or when the traditional non-branched combination was used. The branched model was designed as a framework to interpret simultaneous HR and accelerometry data into minute-by minute physical activity intensity. These authors suggested that individual calibration may not be as necessary when branched modelling is employed. Further, using secondary data analyses (Strath, Brage, & Ekelund, 2005) analysed two recent modelling techniques (Strath, Bassett, Swartz, & Thompson, 2001; Strath, Bassett, Thompson, & Swartz, 2002; Brage et al., 2004) and showed that both were accurate in the prediction of AEE. Johansson et al. (2006) used a similar procedure to the branched equation and showed that the heart rate and accelerometry combination has potential as a method for the assessment of TEE during free-living activities as compared with DLW. A strength of this study was the collection of 14 days of heart rate and accelerometry data however, a limitation was the very small sample size (n=6).

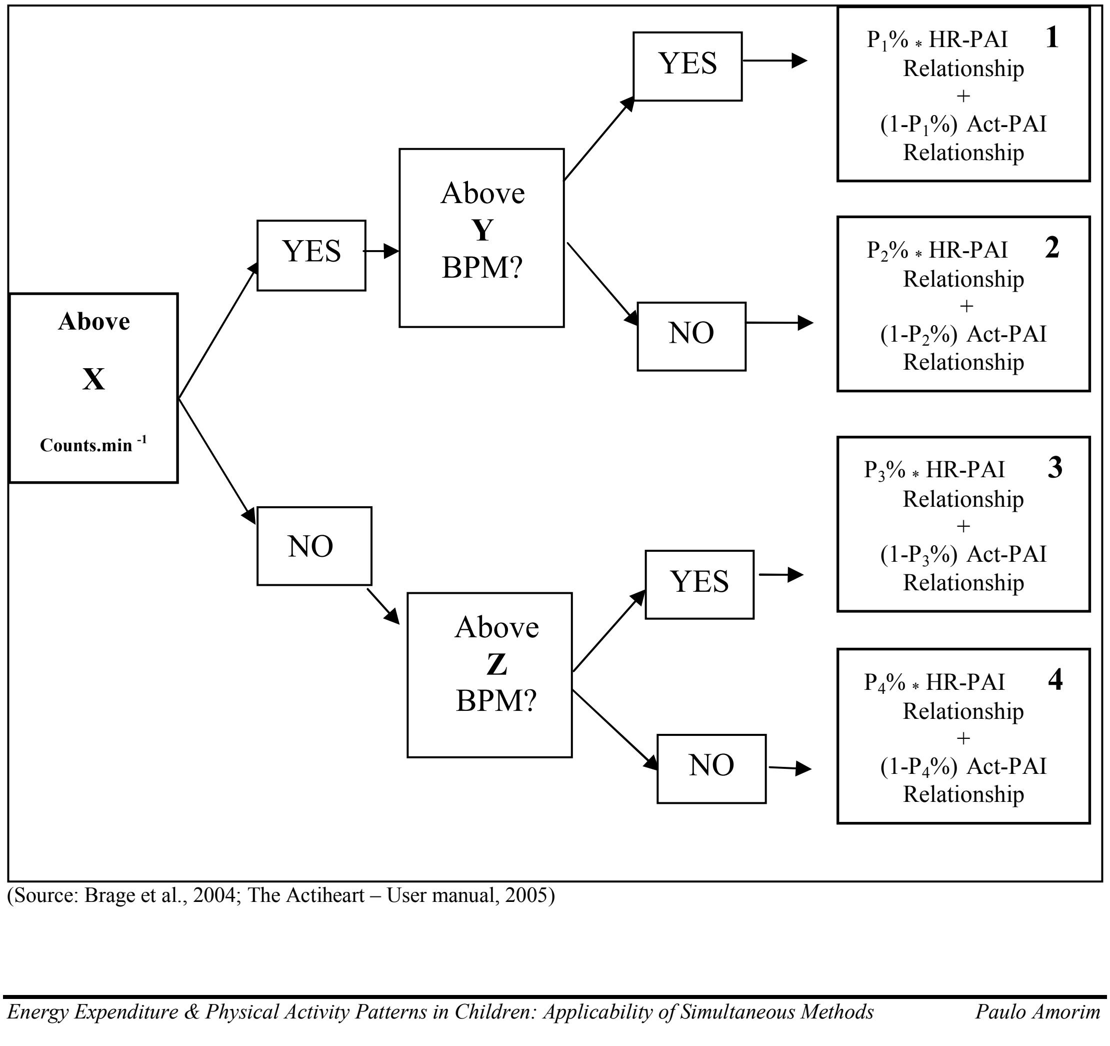
The branched equation model was incorporated into the Actiheart (Cambridge Neurotechnology Ltd., Papworth, UK), a single-piece device combining heart rate and movement sensing. The theory behind the branched model is based on the fact that calibration curves of activity vary due to biomechanical factors and possibly also to the activity mode. In the case of both activity counts and HR there is a point below which the linear relationship no longer holds. This is referred to as the Flex Point and it has been used to demarcate REE from energy expended during physical activity (AEE). If HR is below an individual's Flex-HR point AEE is normally assumed to be zero, the only energy expended being REE. For HR values above Flex-HR, EE is predicted using a regression line. The AEE is set to zero in the classical HR-only model to avoid overestimation of EE in daily living. This does not apply to the branched model shown below due to the low weighting given to HR compared with activity in daily living. Where HR and activity are combined the derivation of the regression line is similar. A multi-linear regression (MLR) equation is derived and expressed in terms of both activity counts and HR.

As shown in Figure 4.1, P1 – P4 are weighting factors. X is used to discriminate between activity and "no-activity". Y and Z are used to apply HR thresholds in the presence and absence of activity, respectively, and Y is used to discriminate between walking and running. At running speeds HR is a very reliable measure of EE whereas activity as measured by vertical acceleration is less reliable since during running the latter does not increase linearly with speed. This is reflected by the weighting in Box 1 where P1 is high. At the other end of the spectrum, HR is a poor measure of intensity whereas movement registration is more reliable and this is reflected by a relatively low weighting of the HR-EE relationship, that is, P4 is low. Z is used to discriminate between elevated HR due to some true activity in the presence of "no activity" (as set by X) and raised HR due to other factors. In boxes 2 and 3, movement and HR are equally weighted.

Brage et al. (2005) have shown that the Actiheart is technically reliable and valid. Corder et al. (2005) completed the first laboratory validation study in children using the device and reported that the monitor is valid to assess AEE during treadmill walking and running. Recently, a study conducted using the Actiheart to verify the precision of different individual calibration procedures showed that a substantial proportion of the between-individual variance in physical activity intensity, accelerometry and heart rate is captured with simple calibration procedures (Brage, Ekelund, Brage, Hennings, Froberg, Franks, & Wareham, 2007). The accuracy of the Actiheart for the assessment of EE in free-living conditions in adults was also recently shown by Crouter et al. (2007) on both a group and an individual basis.

Trost (2001) suggested that if the goal is to estimate energy expenditure, HR monitoring using a HR-$\dot{V}O_2$ calibration curve would be the method of choice, and added that the enhanced precision afforded by the combination of HR monitoring and accelerometry represents an exciting development in youth physical activity assessment.

Figure 4.1 - Branched model combining accelerometry and heart rate



(Source: Brage et al., 2004; The Actiheart – User manual, 2005)

Independent of the selected method to measure physical activity, it is important to consider that each method has strengths and limitations and the validity of each instrument should be analysed. Direct measurement of energy expenditure is only appropriate for small studies because the DLW technique is expensive (Koebnick et al., 2005). Further, indirect calorimetry does not allow assessment in a free-living state without interference to habitual physical activity patterns (Montoye et al., 1996, Molnar and Livingstone, 2000). This is mainly the case for children, despite the advances in technology and the production of a range of portable systems.

The energy expenditure of physical activity may not always be representative of time spent exercising because the daily energy expended in physical activity includes the combined energy cost of all physical activities including sedentary activities (Goran, Hunter, Nagy, & Johnson, 1997). Treuth et al. (1998) using room respiration calorimetry and DLW compared energy expenditure and physical fitness in overweight versus non-overweight prepuberal girls. They found that 24 hr sedentary energy expenditure, TDEE and submaximal $\dot{V}O_{2,}$ after adjusting for FFM or weight and physical activity, energy expenditure and PAL were not significantly different between groups. However, such experimental situations change the environment of the child, and the measurement is not performed under the habitual conditions that would be encountered during the course of a typical day, with usual daily energy expenditure underestimated.

4.2 Indirect techniques based on accelerometry and heart rate to measure energy expenditure: Validation by the doubly labelled water technique.

4.2.1 Introduction

Physical activity is the most variable component of total daily energy expenditure (TDEE) and the many difficulties associated with its measurement relate to methods that are not valid. Since the earlier studies of the effects of physical activity on body weight conducted by Mayer et al. (Mayer, Marshall, Vitale, Christensen, Mashayekhi, & Stare, 1954; Mayer, Roy, & Mitra, 1956) a range of studies of the effects of physical inactivity have suffered from the limitations of the tools used to assess physical activity (Schoeller, 1998).

Sirard and Pate (2001) considered three types of measures of physical activity in children and adolescents: a) Criterion standards or primary measures include direct observation, the DLW technique and indirect calorimetry; b) Secondary measures or objective techniques which have been validated against a criterion standard are heart rate, pedometers and accelerometers; and c) Subjective techniques or survey methods validated against more stringent measures (primary- or secondary-level methods) are self-report questionnaires, interviewer-administered questionnaires, proxy-report questionnaires and diaries.

Energy expenditure, defined as the rate at which heat is produced by the body, is ideally measured by direct calorimetry. Direct calorimetry measures energy expenditure as the rate at which heat is lost from the body to the environment. An individual must be enclosed in a chamber and heat production is measured (Consolazio, Johnson, Daws, & Nelson, 1973). Alternatively, indirect calorimetry estimates heat production using proxy measures, usually a quantitative measurement of the chemical by-products of metabolism. Typically the by-products reflect respiratory gas exchange, like the volume of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), or CO_2 production assessed during the measurement of the excretion of stable isotopes. However, these methods are either invasive, expensive, require complex equipment, or are not designed for children (Sarton-

Miller et al., 2003). Despite expensive, DLW technique has been used in validation studies of other indirect techniques (Koebnick et al, 2005).

Assessing energy expenditure in children performing their common physical activity must occur within the children's usual environment. Such an approach can be challenging as measurements must be taken outside of the laboratory setting. While several methods have been developed to assess energy expenditure in field settings, including the DLW technique and the factorial method; each method has advantages and limitations. The inclusion of techniques such as self-report, direct observation, electronic monitoring, and direct or indirect calorimetry is possible however the application of each of the techniques are quite different (Kohl, Fulton, & Caspersen, 2000). For example, data derived from self-report collection have a relatively low level of precision, whereas laboratory methods such as calorimetry offer greater precision. Because of the differences in precision, the choice of validation standard is a critical aspect of studies of physical activity assessment.

The utilisation of accelerometers, heart rate monitors and the simultaneous use of both with a variety of calibration equations from low to high intensity activities, and considering both upper-body and lower-body activity while controlling for an elevation in HR due to non-exercise reasons by branched equations, has considerable merit. The insertion of filters to clean data and validation against the DLW technique provides an alternative approach to the prediction of energy expenditure as part of an integrated multi-system approach to free-living monitoring in children.

4.2.2 Materials and methods

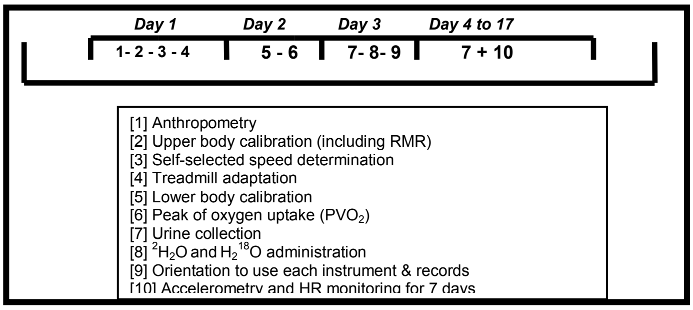
4.2.2.1 Participants

Experiments were carried out on 19 children aged 8-12 years recruited from the Brisbane metropolitan area through advertisements distributed in schools and notes in school newsletters. A sample size calculation to achieve a statistical power of 0.80 at the 0.05 significance level indicated that a sample of 17 participants was required (G*Power 3.0.5 for Windows, 2007). As the main outcome of this study was TEE from DLW measurement, the reference for power calculation data was a meta-analysis of 17 studies of TEE using the DLW technique in children (Hoos et al., 2003), along with recommendations from the *Institute of Medicine* for age and sex used in this research (Brooks et al., 2004). Children were classified as eligible based on age, medical history, ability to tolerate any necessary instrument or apparatus used during testing sessions and monitoring periods, as well as parent's compliance with requirements of the study. Exclusion criteria included individuals with known medical conditions such as type I or type II diabetes mellitus or any metabolic disease. All participants were in good health and were not taking medications known to influence metabolic rate. Participants and their parents or caregivers gave written informed consent that followed the guidelines of the Queensland University of Technology Human Research Ethics Committee.

4.2.2.2 Study Design

On the morning of the first calibration session (between 7:00 and 9:00 am) participants arrived at the exercise physiology laboratory by car having been instructed to remain in an overnight fasted state (no food or drink, except for water) and having abstained from moderate and strenuous exercise in the previous 24 hours. The second calibration session occurred during the afternoon (between 3:30 and 5:00 pm). Participants again arrived at the exercise physiology laboratory by car having been instructed to finish their lunch at least 4 h before the scheduled session time as well as having abstained from moderate and strenuous exercise in the previous 24 hours. When participants had not complied with the required recommendation, measurements were not performed and testing was rescheduled. Each participant completed these two laboratory-based calibration sessions within one week. Thereafter, a week-long free-living monitoring period was carried out in the first week of a 14-day TEE measurement using the DLW technique (Figure 4.2). BMI standard deviation scores were calculated using the percentiles proposed by Cole et al. (2000). RMR was assessed using the methodology described at session 2.2.2 from calibration session 1.

Figure 4.2 – Study design TEE

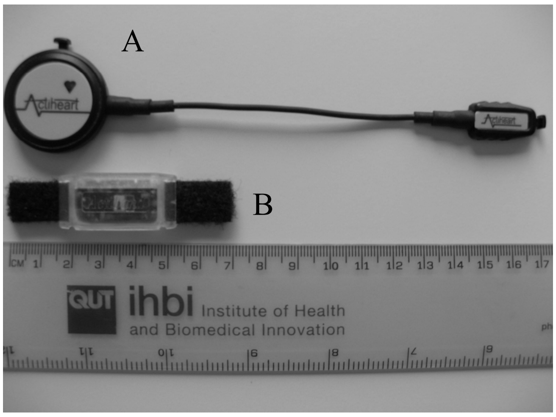


4.2.2.3 **Instrumentation**

The self-selected walking speed (SS) determination and treadmill accommodation were performed using the same procedure as described earlier in the thesis (section 3.2.). Similarly, technical procedures involved in the calibration of instruments were performed as for the earlier study.

Equipment used during laboratory calibration sessions as well as in the 7 day free-living monitoring component of this study to record heart rate (bpm) and activities (cpm), namely Actiheart® (Figure 4.3a) and Actiband® (Figure 4.3b), are described as follows by the manufacturers, Cambridge Neurotechnology Ltd. (Papworth, UK).

Figure 4.3 - Actiheart® and Actiband®



4.2.2.3.1 Actiheart

Technical description

The main component of the Actiheart is 7 mm thick with a diameter of 33 mm and houses a movement sensor, a rechargeable battery, a memory chip, and other electronics (Figure 4.2a). A wire of approximately 100 mm in length runs to a smaller (5 x 11 x 22 mm^3) clip. The total weight of the device is 8 g. The Actiheart is capable of measuring acceleration, HR, HR variability, and ECG amplitude for a set time resolution. Available epoch settings are 15 s, 30 s, or 1 min. The memory capacity of 128 kb allows the user to store data for more than 11 days with an epoch setting of 1 min. Another recording mode stores acceleration (15 s epoch) and all interbeat-intervals (IBI), that is, the time-intervals between ‘R’ spikes of the QRS complex for approximately 24 h, and finally the non-integrated waveforms of acceleration (sampled at 32 Hz) and HR (sampled at 128 Hz) can be recorded for 13 min 38 s. The Actiheart clips onto a single standard ECG electrode with a short ECG lead to another electrode that picks up the ECG signal. It is normally worn on the upper or lower chest.

Movement measurement

Acceleration is measured by a piezoelectric element contained in the Actiheart with a frequency range of 1-7 Hz (3dB). This movement sensor generates a transient charge when exposed to time-varying acceleration and thus produces a voltage signal which is then converted into a binary signal by an 8-bit A/D converter. This results in 256 distinctive levels of acceleration (128 positive and 18 negative levels). The accelerometer has a dynamic range of ±25 m.s^{-2} (±2.5 G) and its sensitivity per bit is 0.2 m.s^{-2} (0.02 G). The instantaneous acceleration is quantified as the numerical difference from zero acceleration in binary units, thus leaving a 2-bit (~0.4 m.s^{-2}) wide deadband. The binary signal is stored in a cache 32 times a second and summed up over the epoch. At the end of the epoch, the sum is divided by 16 and then again by 2, N numbers of times until the number is below 32. The resulting integer and N are then stored in a single byte (5 bits for integer, 3 bits for N) in the non-volatile memory and the cache is reset to zero. The movement sums are then divided by the calibration factor for the particular Actiheart unit during the download by the software. Actiheart units are calibrated by the manufacturer with sinusoid accelerations of ±1 G (average ~0.7 G), obtained with a calibration frequency of 3 Hz.

Heart rate measurement

The actiheart has a sensitivity of 0.250 mV. The ECG signal is electronically amplified by a factor of 900 (amplifier frequency response: 10-35 Hz). The resulting ECG signal is sampled at 128 Hz and each R-wave decaying edge is identified by using the average difference of ECG samples n_i and n_{i+1} and ECG samples n_i and n_{i+2}. The threshold detection sensitivity changes with the amount of movement detected. At the end of the epoch, the trimmed mean of the last 16 R-R intervals is calculated by ignoring values outside ±25% of the initial mean. This is converted to beats-per minute (bpm) and written to the memory at the end of each epoch. When the Actiheart is set up to record HR variability over the epoch, the two fastest and the two slowest interbeat intervals occurring in that epoch are also stored. The measurable range of HR in the manufacturer specification is 31-250 bpm.

Positioning

The Actiheart was placed with the cable exit as near the horizontal as possible to maximise the accuracy of the measurements. The electrodes were positioned so that the cable was stretched to its full length to avoid unnecessary rotation of the sensor in situ (see Figure 4.4):

Figure 4.4 – Actiheart positioning

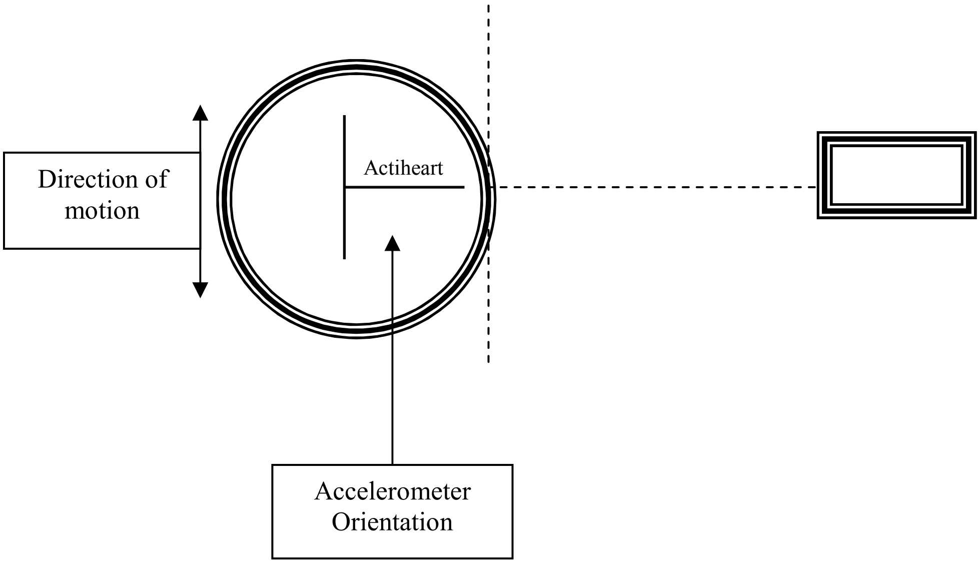


Table 4.1 below summarises the recording durations.

Table 4.1 - Actiheart record duration

Epoch	15 sec	30 sec	1 min
Recording time HR+Act	10 days	20 days	21 days
Recording time HR+Act +IBI Max min	2.5 days	5 days	11 days
Recording IBI 120,000 beats maximum.	24 hrs		

4.2.2.3.2 Actiband

Technical description

The Actiband is a lightweight, waterproof device which can be worn on the wrist, leg or waist and records physical activity and steps counts (Figure 4.3b). The user has the ability to select the epoch length required for the recording. This can be 15 second, 1 minute or Waveform. Waveform recording mode records the complete analogue waveform for up to 33 minutes. The Actiband contains a rechargeable battery and a memory capacity of 64 kb providing up to 90 days of continuous recording. It is easily recharged via the purpose built interface that links to a computer USB port. The same interface is used for downloading the data to a PC where the data is analysed using the Actiband software.

Movement measurement

Waist and ankle

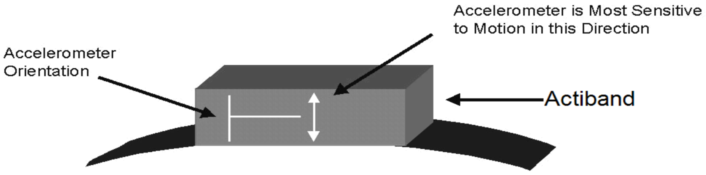
Acceleration is measured by a piezoelectric element contained in the Actiband with a frequency range of 1-7 Hz (3 dB). This movement sensor generates a transient charge when exposed to time-varying acceleration and thus produces a voltage signal which is then converted into a binary signal by an 8-bit A/D converter. This results in 256 distinctive levels of acceleration (128 positive and 128 negative levels). The accelerometer has a dynamic range of ±25 $m.s^{-2}$ (±2.5 G) and its sensitivity per bit is 0.2 $m.s^{-2}$ (0.02 G). The binary signal is stored in a cache 32 times a second and summed up over the epoch. An epoch period of 1 min would thus comprise the sum of 1920 samples of the acceleration signal. At the end of the epoch, the sum is divided by 16 and then again by 2, *N* numbers of times until the number is below 32. The resulting integer and *N* are then stored in a single byte (5 bits for integer, 3 bits for *N*) in the non-volatile memory and the cache is reset to zero. The sum is restored to its original value when the data is read into the computer. The accelerometer is designed to detect vertical movement with the person upright.

Wrist

Wrist-mounted devices have some technical differences to waist and ankle-mounted devices as shown: Range: > ±5 G and Frequency range: 3 Hz to 11 Hz

The electronics in the watch checks or samples the amplitude thirty two times per second and it captures the highest amplitude in each second which represents the peak intensity of the movement. The peaks in an epoch (15 or 60 s) are summed and stored.

Figure 4.5 – Actiband schematic representation



Actiband mounting positions

The Actiband is available with 3 different mounting positions as follows:

Wrist

The Actiband can be worn on the wrist using a standard strap. Step counting is not possible in the wrist mode.

Waist

The Actiband can be worn mounted to a waist band and in this position the step counter is enabled.

Ankle

An ankle strap enables the unit to be mounted on the ankle where it operates in the same way as the waist–mounted unit.

The recording time and epoch lengths available are dependent on the mounting position selected. This is summarised in the following table.

Table 4.2 - Actiband record duration

Mounting position	15 sec	1 min	Waveform
Wrist	10 days	42 days	33 min
Waist	5 days	21 days	33 min
Ankle	5 days	21 days	33 min

This was the first time that Actiband accelerometers were applied outside the manufacturer's laboratory. To verify the Actiband sensitivity to speed changes a treadmill test was conducted with 4 walking (1.6 and 0.8 km.h^{-1} less than SS and 0.8 km.h^{-1} faster than SS) and 2 running speeds (1.6 and 2.4 km.h^{-1} faster than SS).

4.2.2.3.3 Individual calibration and use

Each participant was individually-calibrated under standardised conditions to establish the HR-$\dot{V}O_2$ and CPM-$\dot{V}O_2$ relationship. In the first calibration session participants were fitted with Actiheart (HR and accelerometry – chest mounted) and 1 Actiband (Accelerometry – wrist mounted). During a second calibration session participants were fitted with Actiheart (HR and accelerometry – chest mounted) and 3 Actibands (Accelerometry – waist, wrist and ankle mounted). Serial numbers of each device were recorded during calibration sessions to ensure that the same device was used for each participant during the monitoring period. Calibration points were obtained by simultaneous measurement of HR-CPM and $\dot{V}O_2$ for the following activities in 2 sessions.

Session 1: [Upper body calibration (UB)] – After a 20 min stabilisation period activities were carried out in 10 min sequences: supine (TV watching), sitting (TV watching), sitting playing computer games, and completing a sub-maximal arm ergometer test (Monark Rehab Trainer 881E). The protocol was continuous with workload increased each minute. The initial cadence was 40 rpm, and increased by 10 rpm every minute. Initial resistance was set as 0 for the first 2 min. Thereafter, resistance was increased by 5 W for the next 3 min. Subsequently, cadence remained constant at 70 rpm and resistance increased by 5 W every minute until participants reach 70-75% of their age-predicted maximal HR, or they requested to stop.

Session 2: [Lower body calibration (LB)] – After 5 min standing, participants started walking continuously on a treadmill at 2 slower speeds (1.6 and 0.8 km.h^{-1} slower than SS), SS, and a faster speed (0.8 km.h^{-1} faster than SS). Speed increments occurred at the 3 min stage. At the end of the faster walking speed stage increments of 0.8 km.h^{-1} occurred each 1 min for the next 2 min. Thereafter, an elevation of 2%

in the treadmill gradient was applied at 1 min intervals until participants reached 80-85% of their age-predicted maximal HR, or they requested to stop.

Heart rate and accelerometer monitoring in free-living activities

Detailed instructions were discussed with each child and their parent before starting the monitoring period and continuous reinforcement was provided by SMS messages, phone calls and daily e-mails during the 7 day monitoring period. Free-living general instructions and a "time not worn log" can be seen in Appendix 7. Each child was fitted with the Actiheart (chest) and 3 Actibands (waist, wrist and ankle) instrumentation early in the morning and removed by the parent at bedtime. Only on the first day were children required to wear the Actiheart during sleep time to determine 'sleep heart rate'.

Accelerometers were fixed to a specific elastic belt for both ankle and waist. The wrist accelerometer has a built-in plastic band similar to a regular watch. All devices were synchronised to the same PC stopwatch to ensure that all information was collected simultaneously.

4.2.2.4 Doubly-labelled water technique (DLW) – the gold standard for TEE measurement

Measurement of ^{2}H and ^{18}O enrichment in urine

The abundance of ^{2}HOH and $H_2{}^{18}O$ in urine, the dilution of the dose aliquot and tapwater used to make the dilution was measured by continuous flow-isotope ratio mass specrtometry (Hydra 20-20, SerCon Ltd, Crewe, UK) against gravimetrically prepared standard waters calibrated against the international standard for ^{18}O and ^{2}H (Vienna Standard Mean Ocean Water, VSMOW). All samples were analysed in triplicate so that the reliability of the measurement could be assessed for each sample.

Analysis of ^{2}H abundance. An aliquot (0.5 mL) of each sample was transferred to a 10 ml Exetainer gas testing vial (Labco, High Wycombe, UK). Glass inserts (200 µl, Chromacol, Welwyn Garden City, UK) containing platinum catalyst (platinum 5 % on alumina powder, -325 mesh, surface area > 250 $m^2.g^{-1}$, Sigma Aldrich, Gillingham, UK) were added to each vial, taking care not to wet the catalyst.

Exetainer vials were filled with hydrogen reference gas and left to equilibrate at ambient temperature, for 48 h prior to analysis. The abundance of deuterium in the gas phase was measured by CF-IRMS (Scrimgeour, Rollo, Mudambo, Handley, & Prosser, 1993). The abundance of ^{2}H in samples was calculated with reference to the known abundance of reference waters, placed at intervals in each batch of samples, and drift corrected between references. Results were expressed in delta units (δ ‰, delta per mil, parts per thousand) relative to the international standard, VSMOW. The enrichment of ^{2}H was calculated by subtracting the measured abundance of the baseline sample from that of the post-dose samples.

Analysis of ^{18}O abundance An aliquot (0.5 ml) of each sample was transferred to a 10 mL Exetainer gas testing vial (Labco, High Wycombe, UK). Vials were filled with CO_2 reference gas and left to equilibrate with the headspace gas for 24 h at ambient temperature. The abundance of ^{18}O in the gas phase was measured by CF-IRMS, monitoring *m/z* 44 and 46. The abundance of ^{18}O in samples was calculated with reference to the known abundance of reference waters, placed at intervals in each batch of samples, and drift corrected between references. Results were expressed in delta units (δ ‰, delta per mil, parts per thousand) relative to the international standard, VSMOW. The enrichment of ^{18}O was calculated by subtracting the measured abundance of the baseline sample from that of the post-dose samples.

Calculation of TBW (body composition) and TEE

The natural logarithm of sample enrichment was plotted against time to provide regression lines. The gradients of the regression lines are the disappearance rates for ^{18}O (ko) and ^{2}H (kd). The dilution space (N_x, kg) of each isotope is calculated from the measured abundance of the 4 and 6 hour urine samples, and the analysis of the diluted dose (DD) and tapwater used to make the dilution.

$$N_x\ (\mathrm{kg}) = (TA/a) \times (\Delta DD/\Delta BW)$$

Where *T* the amount of tap water in the diluted dose (g)

A is the amount of DLW consumed by the participant (g) and *a* is the amount of dose in the diluted dose (g).

ΔDD is the isotopic enrichment of the diluted dose i.e. the measured abundance in the diluted dose minus the measured abundance in the tapwater.

ΔBW is the isotopic enrichment of body water i.e. the measured abundance in the post dose urine sample minus the measured abundance in the baseline urine sample. N_h is the ^2H dilution space and N_o is the ^{18}O dilution space.

Total body water (TBW, kg) is calculated from N_x by dividing by the appropriate non-aqueous exchange factor (1.04 for ^2H and 1.01 for ^{18}O).

$TBW_h = N_h/1.04$ $\qquad$ $TBW_o = N_o/1.01$

TBW_{avg} is the mean of TBW_h and TBW_o

Calculation of body composition

Fat free mass (FFM, kg) = TBW (kg)/hydration coefficient for FFM. In adults this is 0.732. In children FFM contains more water than adults (Lohman, 1986). The appropriate coefficient should be used depending on the age and sex of the child (Table 4.3).

Hydration of fat free mass in children (%)

Table 4.3 – FFM hydration coefficient for children

Age (years)	Male	Female
1	79.0	78.8
1-2	78.6	78.5
3-5	77.8	78.3
5-6	77.0	78.0
7-8	76.8	77.6
9-10	76.2	77.0
11-12	75.4	76.6
13-14	74.7	75.5
15-16	74.2	75.0
17-20	73.8	74.5

From: Lohman (1992)

Fat mass (kg) = Body mass (kg) – FFM (kg)

Body fat (%) = Fat mass (kg)/body mass (kg) x 100

Calculation of TEE

TBW_{avg} (kg) is converted to TBW (mol) by dividing by the molecular weight of water (18.0153).

CO_2 production rate (rCO_2, moles/day) is calculated from TBW (mol) after correction for fractionation of water leaving the body in breath and transdermal evaporation (Coward, 1988).

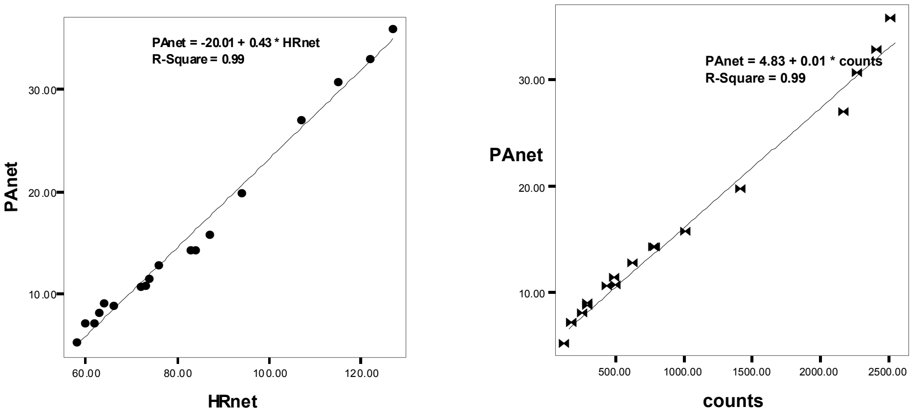
CO_2 production rate (mol/day) is converted to L/day by multiplying by 22.414
Oxygen consumption rate is calculated assuming RQ = 0.85
rO_2 (L/day) = rCO_2 (L/d)/0.85
TEE is calculated using the Weir (1949) equation
TEE (kcal/d) = (3.941 x rO_2 (L/d)) + (1.106 x rCO_2 (L/d))

4.2.2.5 Estimation of energy expenditure by heart rate and accelerometers

Considerable variation exists in the relationships between activity intensity and HR and accelerometry, which may be reduced by individual calibration. Using data from calibration sessions, twelve different individual procedures were developed (Table 4.4) to estimate AEE and TEE from 7 days free-living monitoring data and further applied against a reference method.

Examples of two individual calibration equations based on HR_{net} [HR exercise minus sleep HR (SHR)] and counts against PA_{net} (measured PA exercise minus measured RMR) are shown below (Figure 4.6).

Figure 4.6 – Individual calibration equation HR and counts



Calibration regression equations were derived from laboratory calibration for LB (treadmill exercise-based) and UB (arm ergometer-based). A HR-$\dot{V}O_2$ regression equation for each exercise type was developed and based on a ratio between accelerometer data mounted at wrist and ankle (wrist$_{cpm}$ divided by ankle$_{cpm}$) to verify movement predominance, one or another equation was chosen. It has been shown that a ratio of greater than 25 between arm and leg activity accurately reflects measured EE when using the simultaneous HR+accelerometer technique (Strath et al., 2001, 2002, 2005). A ratio of greater than or equal to 25 was indicative of arm work (UB activity predominance), whereas a ratio of less than 25 was indicative of leg work (LB activity predominance). HR-$\dot{V}O_2$ relationship for leg activity closely represents combined arm-and-leg activity (Haskell et al., 1993). To reduce participant burden by the continuous use of elastic bandage on the thigh for an extended period of time, as well as for increased compliance with the exact device location, leg work was measured with accelerometer located at ankle. For all accelerometer-based equation was used LB calibration (CPM-$\dot{V}O_2$ regression).

Energy expenditure for periods of daytime when HR or CPM regression equations exhibited values lower than RMR or the mean of resting $\dot{V}O_2$ for 4 resting activities [(4act) = supine watching TV, sitting watching TV, sitting playing computer games and standing], dependent on the used procedure, this value was replaced by the RMR value or 4act and referred to as resting energy expenditure (REE). The inclusion procedure of 4act was chosen because these activities are representative of low intensity activities habitually undertaken by children daily. For the remainder of the time, EE was calculated from the minute-by minute recorded HR and CPM and each participant's calibration curves for upper or lower body activities and referred to as activity energy expenditure (AEE). Energy expenditure during sleep (SEE) was calculated by the formula: nightly EE = REE – REE/10 (Beghin et al., 2000). Twenty-four hour TEE was computed by summing the REE, AEE and SEE. The monitoring period was proportionally adjusted for 14 hours of non-sleep time, estimated for this population based on a sleep time survey recently conducted in Australia (Dollman et al., 2007) and using the following equation from Andersen et al. (2006):

Adjusted minutes = observed in interval X (14 X 60/total minutes)

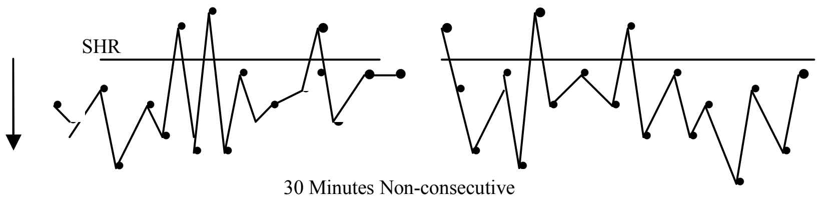
This procedure allows to be more consistent than using only raw average times considering between participant comparisons.

Table 4.4: Procedures to estimate AEE and TEE

Calibration	Description
$HRUBLB_{RMR}$	Wrist/ankle ≥ 25 = UB equation, < 25 = LB equation, if < RMR = RMR
$HRUBLB_{4act}$	Wrist/ankle ≥ 25 = UB equation, < 25 = LB equation, if < 4act = 4act
$HRLB_{4act}$	Only LB equation, if < 4act = 4act
AH_{cpm}	AH_{cpm} < 4act = 4act
AB_{Waist}	AB_{Waist} < 4act = 4act
AB_{ankle}	AB_{ankle} < 4act = 4act
AB_{Wrist}	AB_{Wrist} < 4act = 4act
$FlexHR_{UBLB}$	HR > FlexHR: Wrist/ankle ≥ 25 = UB equation, < 25 = LB equation, if HR < FlexHR = 4act
$AH_{branched}$	AH branched equation using built-in group equation for children
$HRPAnet_{RMR}$	PA_{net} and HR_{net}. Wrist/ankle ≥ 25 = UB equation, < 25 = LB equation, if < RMR = RMR
$HRPAnet_{4act}$	PA_{net} and HR_{net}. Wrist/ankle ≥ 25 = UB equation, < 25 = LB equation, if < 4act = 4act
$HRPALBnet_{4act}$	PA_{net} and HR_{net}. Only LB equation, if < 4act = 4act

To calculate sleep heart rate (SHR) the Actiheart was worn for one complete night. The SHR is set at the highest value of the thirty lowest minute-by minute HR readings during a 24 hr day, as exemplified in figure 4.7.

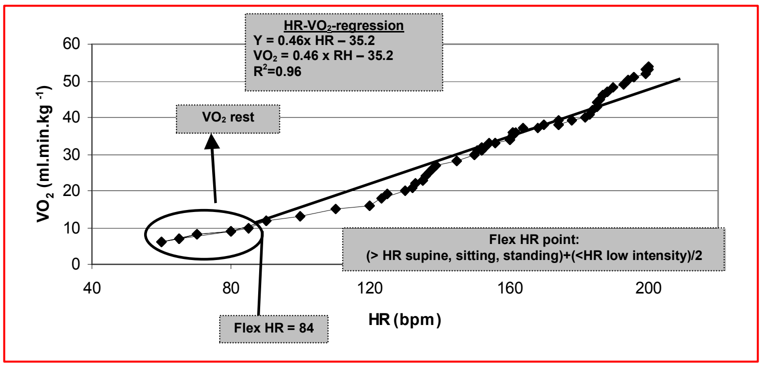
Figure 4.7: Example of SHR set by AH software



(Source: The Actiheart – User manual, 2005)

Flex-HR was calculated as the mean of the highest HR for the resting activities and the lowest HR of the exercise activities (Livingstone et al., 1992; 2000), as shown in figure 4.8.

Figure 4.8: FlexHR point determination



4.2.2.5.1 HR data cleaning

Before estimation of TEE, HR data was filtered. The continuity of the HR record is occasionally broken, due either to loss of electrode contact or the introduction of spuriously high values due to electrical interference. One of the useful features of Actiheart is the capability regarding data cleaning, removal and replacement. Lost or corrupt HR data will affect the calculation of TEE. There are three steps in the cleaning process and this involves setting suspect values to zero according to the following criteria:

HR < 30 or

HR > 30 and the rate of change of heart rate is > 100 bpm for a 1 min epoch.

Step 1: The cleaned data is analysed minute-by minute and if the cleaned HR at any data point is >1.75 * Filtered HR then the cleaned HR is set to zero. Filtered HR is the average of the HR over the 4 minutes preceding the data point being analysed.

Step 2: For each minute where the HR has been set to zero each stored IBI minimum and maximum is used to calculate a HR (60,000/mS) which is then compared with the previous valid heart rate. The calculated HR which is closest to the valid heart rate and within 30 bpm is used to replace the zero values. This is termed “Recovered

Data”. If the difference is > 30 bpm the calculated HR is not used to replace the zero value.

Step 3: Following the recovery process any remaining zero value gaps of < 5 minutes are filled by interpolation (straight line join). If the gap is greater than 5 minutes the value is left at zero.

This cleaning process has been supported in the literature. For example, Wareham et al. (1997) recommended that if more than five aberrant readings occur in succession, the data should not be interpolated but rather the segment has to be removed. The insertion and filtering of the HR data has been shown to make a substantial improvement to the HR-EE relationship (Davidson et al., 1997).

4.2.2.5.2 Accelerometer data cleaning

We have analysed our data for each accelerometer considering the information in the “not worn time log” checked against accelerometer CPM and those periods of repeated zero counts corresponding to the records were excluded. Continuous 10 min periods of zero records which occurred simultaneously in every accelerometer and not specified in the “not worn time log” were also excluded.

To enable further comparison with other studies, a new set of data were constructed and analysed using the waist-mounted accelerometer output as a reference. Information noted in the “not worn time log” were checked against accelerometer CPM and all continuous 10 min periods of zero records at the waist-mounted accelerometer matched with the “not worn time log” notation were excluded. Following the same procedure, but this time independent of the “not worn time log” notations, all 10 min periods of continuous zero registered by the waist-mounted accelerometer were also excluded. Finally, if continuous 10 min periods of zero records were verified simultaneously in all accelerometers, these periods of time were also excluded.

4.2.2.5.3 Time window analyses

School days were divided into four time windows:

Block 1: from 08:31 am to 12:00 midday
Block 2: from 12:01 pm to 15:30 pm
Block 3: from 15:31 pm to 19:00 pm
Block 4: from 15:30 pm to 17:30 pm

Block 3 and 4 are from similar periods of the day and were chosen to allow meaningful comparisons with the literature where the 2 hours after school are considered. In some analyses, to allow comparisons among all time windows, block 4 was adjusted by the equation from Andersen et al. (2006), as previously cited.

Analyses in these time windows were performed considering HR techniques for AEE estimates as well as for accelerometer counts. MBPA (mean block physical activity) was calculated for each accelerometer (total CPM in each block divided by number of minutes), to facilitate comparisons between them. Additionally, the sum of all accelerometers MBPA was considered in each block.

Our design using a multi-accelerometer system allowed us to verify the relative participation of each body segment for the total block count in each time window using the following equation:

Body segment % = $(\text{Accel}_{\text{C, WA, WR or AN}}\ \text{MBPA} * 100/\ \text{sum of}_{\text{C, WA, WR, AN}}\ \text{MBPA})$

Where: C = Chest, WA = waist, WR = wrist and AN = ankle

4.2.2.5.4 Cross-validation

A group equation, laboratory-derived using the treadmill exercise from participant's data described in section 3.3 to predict AEE, was cross-validated against laboratory-measured AEE in the study sample, under the same controlled conditions. Further, this equation was applied to free-living conditions data to predict AEE and TEE.

4.2.2.5.5 Statistical analyses

Data is presented as mean values and standard deviations (SD), unless otherwise stated. The agreement and relative bias between estimated TEE from each of the 12 procedures and the DLW technique was assessed using the method of Bland and Altman (1986). To verify accelerometer counts against all six speeds, repeated measures ANOVA was used. Day-of-week differences were assessed by *T*-test. TEE reliability between–day was assessed using intraclass correlation coefficients (ICC). To analyse within-day and between-day variability, coefficients of variability (CV) were determined. Pearson correlation coefficients were computed to determine the linear relationship between activity counts and HR techniques and TEE and AEE as well as between activity counts and HR techniques and physiologic variables. Partial correlations were computed to determine the relationship between activity counts and HR techniques and TEE and AEE adjusted for physical characteristics variables. Stepwise multiple regression analysis was used to determine which of the independent variables contributed to the variation in TEE and AEE. To test the validity of the equation used in cross-validation sessions, the degree of agreement between predicted and DLW measured AEE and TEE were assessed by the method of Bland and Altman (1986). Values were considered significant at $P < 0.05$. Statistical analyses were carried out with SPSS for Windows (version 15.0, 2006, SPSS, Chicago, IL, USA).

4.2.3 Results

Age, anthropometric characteristics, body fat and fat free mass (FFM) of the sample are presented in Table 4.5.

Table 4.5 – Sample characteristics

Age (years)	Weight (kg)	Height (cm)	Body fat* (%)	FFM# (kg)
10.3±1.0	37.0±5.9	143±9.0	28.9±8.0	25.9±3.0

* Deuterium technique # Hydration coefficient from Lohman (1992)

4.2.3.1 Accelerometers and speed detection

The ability of accelerometers to detect speed changes was verified and average speeds were: V1 = 3.4 $km.h^{-1}$; V2 = 4.2 $km.h^{-1}$; V3 = 5.0 $km.h^{-1}$; V4 = 5.8 $km.h^{-1}$; V5 = 6.6 $km.h^{-1}$ and V6 = 7.5 $km.h^{-1}$ [SD = 0.3 for all speeds as speeds were determined from SS (V3)]. AH_{cpm}, $ABwaist_{cpm}$ and $ABankle_{cpm}$ showed statistically significant differences ($p<0.001$) for all 4 walking speeds. For the 2 running speeds, AH_{cpm} and $ABwaist_{cpm}$ showed a statistically significant difference ($p<0.001$). Accelerometer cpm and speed relationship are depicted graphically in figures 4.9, 4.10 and 4.11, respectively for AH_{cpm}, $ABwaist_{cpm}$ and $ABankle_{cpm}$. Pearson's correlation among accelerometers cpm and the physiologic criterion variables were statistically significant ($p<0.01$) for all analysed variables (Table 4.6).

Table 4.6 – Accelerometer cpm and correlations with physiologic criterion variables

Variables	AH cpm	AB_{waist} cpm	AB_{ankle} cpm
$EE_{kcal.min^{-1}}$	.63	.60	.55
$\dot{V}O_2$ $ml.kg^{-1}.min^{-1}$	.81	.81	.42
HR_{bpm}	.74	.76	.45
$Speed_{km.h^{-1}}$	.86	.86	.60

All significant ($p<0.01$)

Figure 4.9 - AH_{cpm} and speed

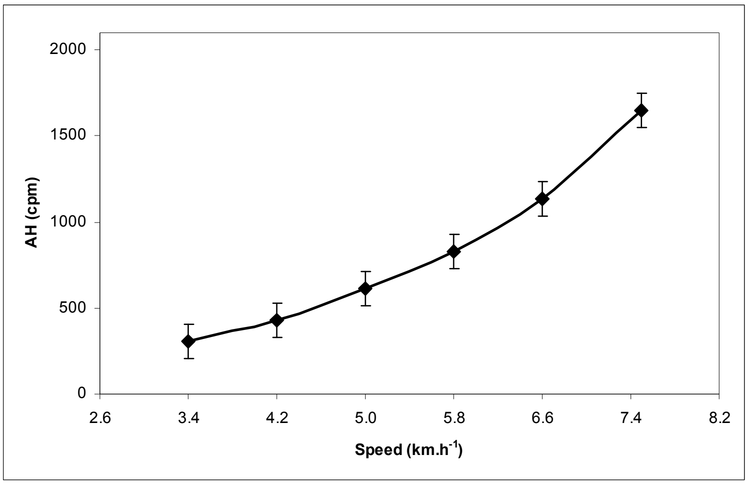


Figure 4.10 - AB $waist_{cpm}$ and speed

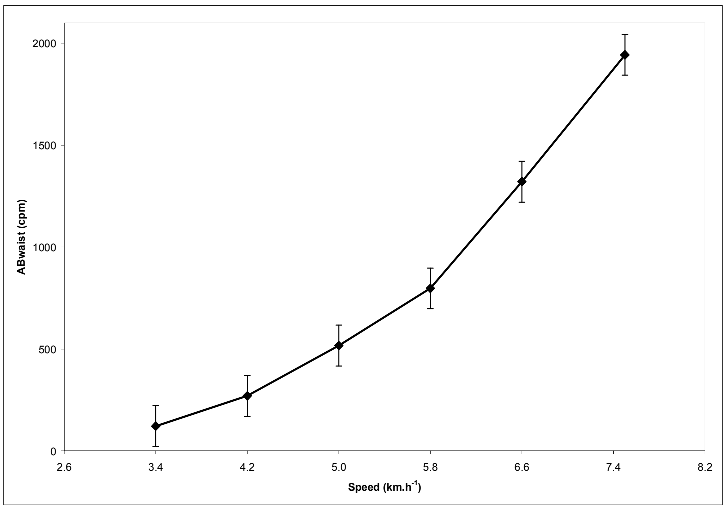
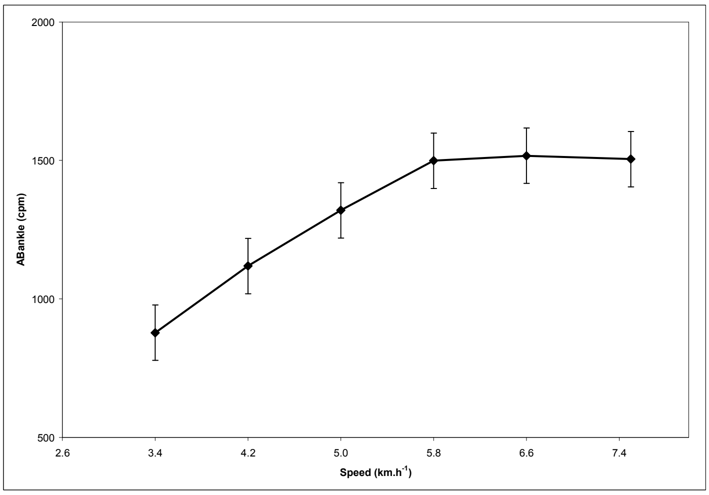


Figure 4.11 - AB $ankle_{cpm}$ and speed



4.2.3.2 Total energy expenditure

TEE DLW, RMR and physical activity level (PAL = TEE/RMR) of the sample are presented in Table 4.7.

Table 4.7 – Sample TEE, RMR and PAL

TEE DLW ($kcal.d^{-1}$)	RMR ($kcal.d^{-1}$)	PAL
2194±389	1209±212	1.86±0.43

PAL was calculated for each participant and sample distribution for PAL categories is shown in Table 4.8.

Table 4.8 – Sample physical activity level characterisation

PAL categories	PAL range[1]	Sample distribution
Sedentary	1.00 to 1.39	2 (10.5%)
Low active	1.40 to 1.59	3 (15.9%)
Active	1.60 to 1.89	7 (36.8%)
Very active	1.90 to 2.50	7 (36.8%)

[1] Institute of Medicine Report, 2002, 2004

The mean number of days monitored was 6.1±1.3 days and mean number of hours of monitoring per day was 12.1±1.4 hours. The mean total hours of monitoring per participant were 72.5±2.2 h.

The degree of agreement between DLW_{TEE} and all other techniques used to predict TEE and AEE tested by the Bland and Altman (1986) method are displayed in the following figures (Figures 4.12 to 4.20). The gray dotted lines represent the tendency line and the black lines represent 95% confidence intervals. Only techniques where the mean of the differences were not significantly different from zero are exhibited.

Figure 4.12 - DLW_{TEE} - $HRUBLB_{RMR}$

Mean difference: 140.2 95%CI: -80.5 to 361.0 SEE: 104.6

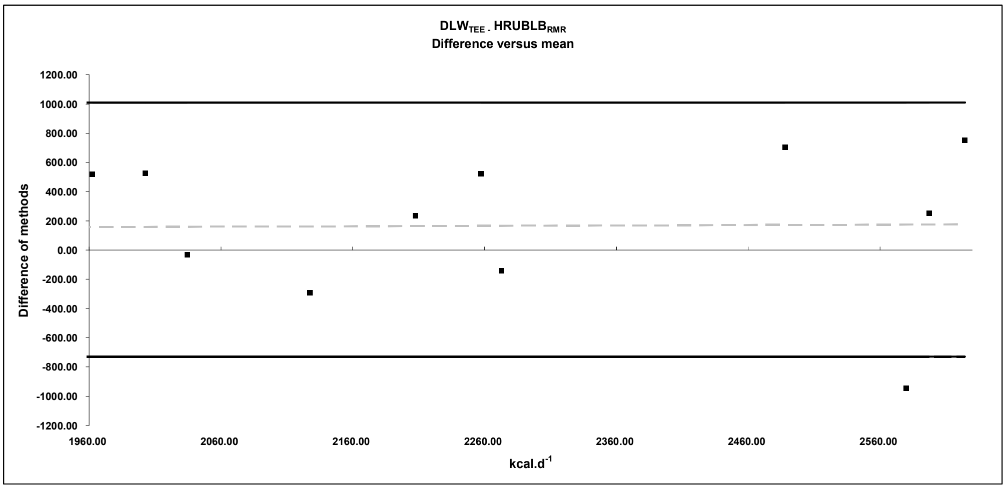


Figure 4.13 - DLW_{TEE} - $HRUBLB_{4act}$

Mean difference: 136.4 95%CI: -79.3 to 352.0 SEE: 102.2

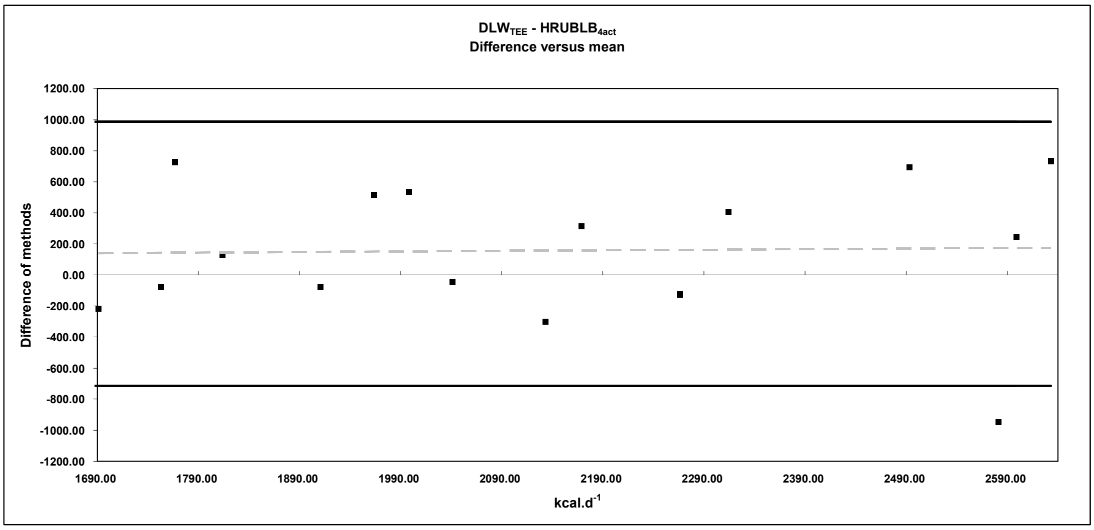


Figure 4.14 - DLW_{TEE} - $HRLB_{4act}$

Mean difference: 118.2 95%CI: -105.3 to 341.7 SEE: 105.9

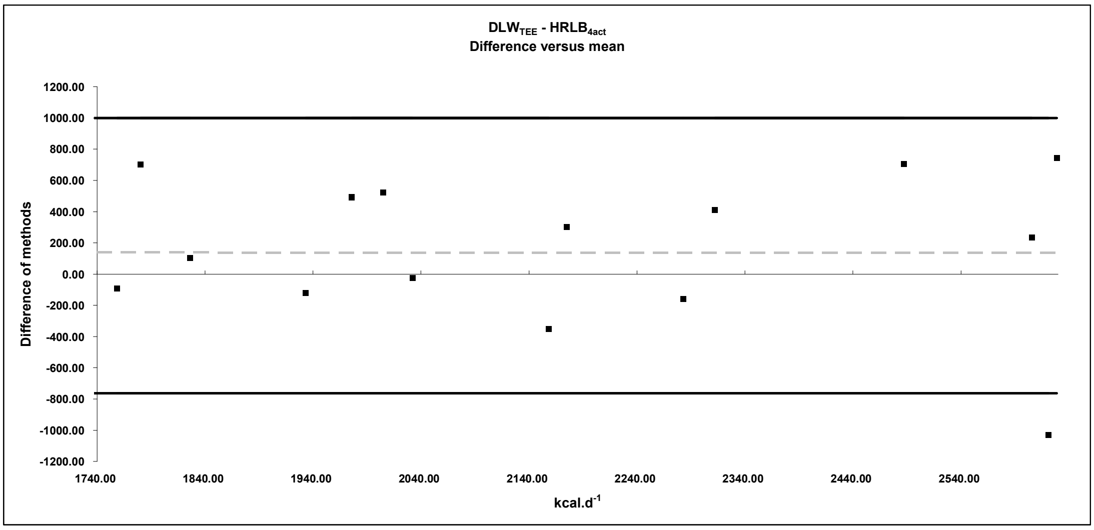


Figure 4.15 - DLW_{TEE} - AH_{cpm}

Mean difference: 194.2 95%CI: -46.4 to 434.8 SEE: 114.0

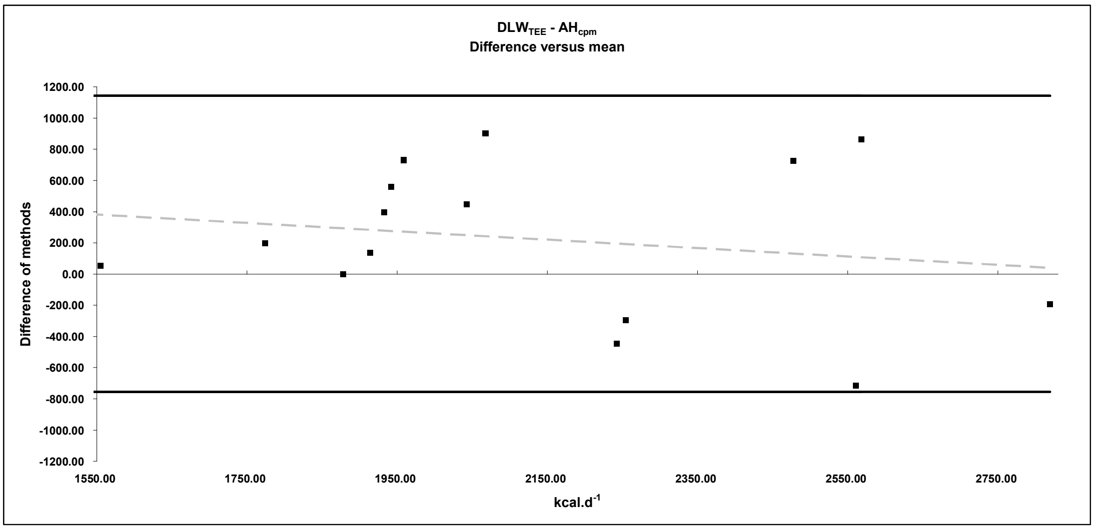


Figure 4.16 - DLW$_{TEE}$ - AB$_{waist}$

Mean difference: -161.9 95%CI: -410.1 to 86.4 SEE: 117.7

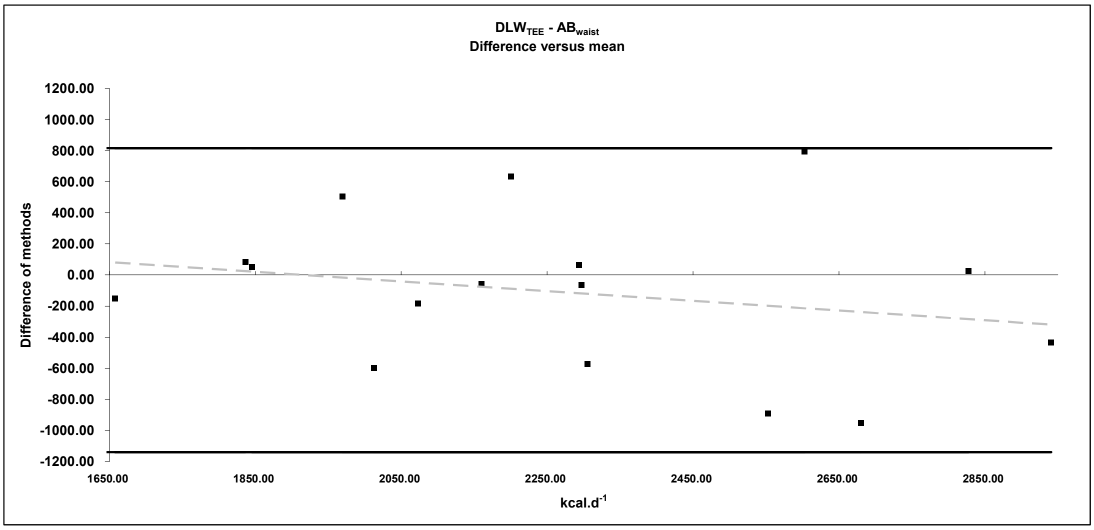


Figure 4.17 - DLW$_{TEE}$ - AH$_{branched}$

Mean difference: -72.0 95%CI: -238 to 93.9 SEE: 79.0

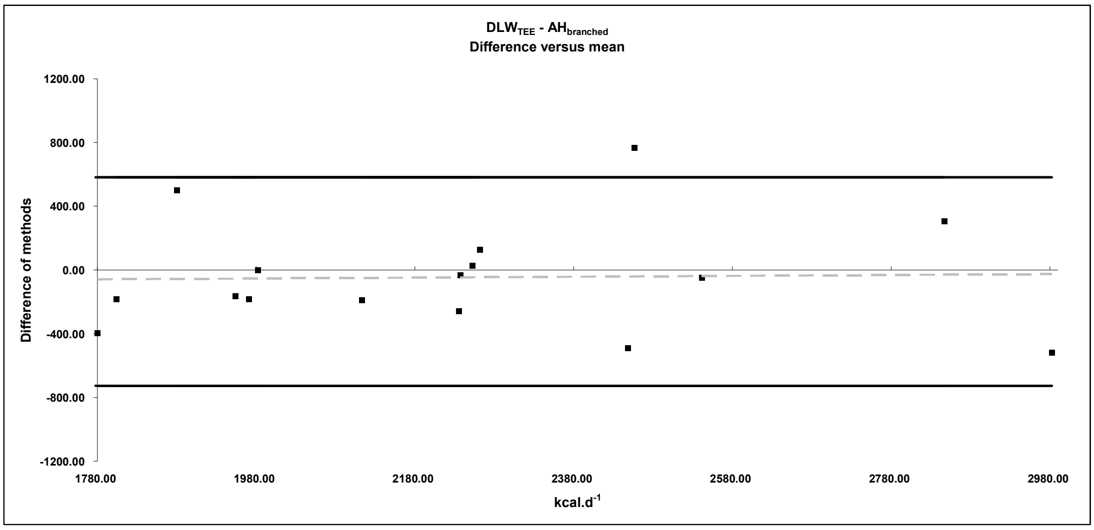


Figure 4.18 - DLW$_{TEE}$ - HRPAnet$_{RMR}$

Mean difference: -67.3 95%CI: -276.6 to 141.9 SEE: 99.2

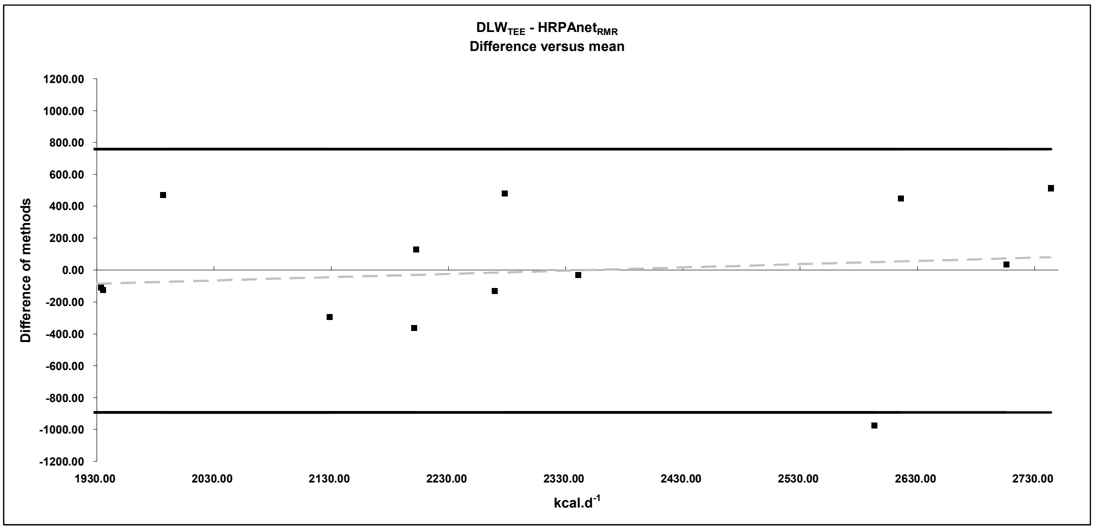


Figure 4.19 - DLW$_{TEE}$ - HRPAnet$_{4act}$

Mean difference: -108.6 95%CI: -319.6 to 102.4 SEE: 99.9

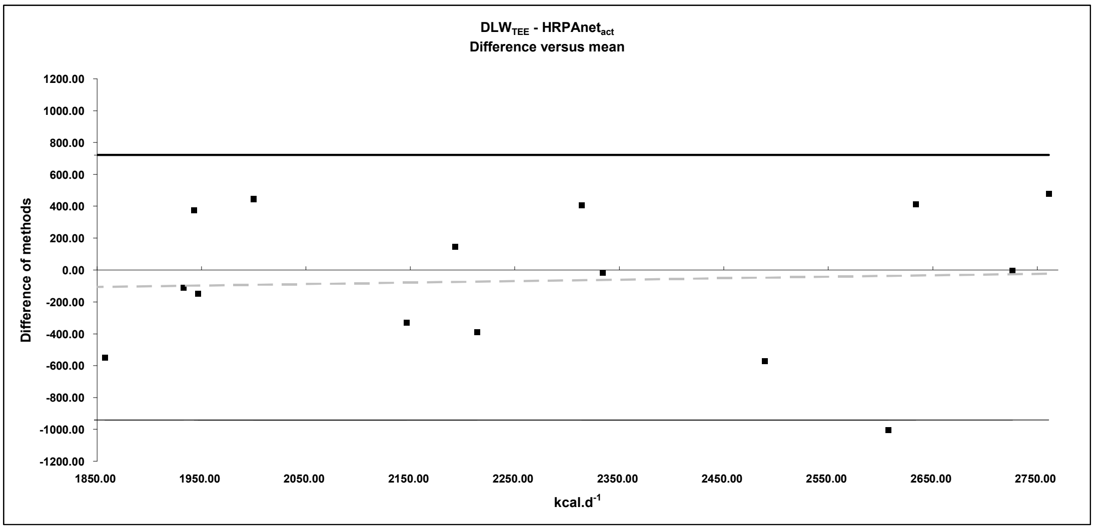
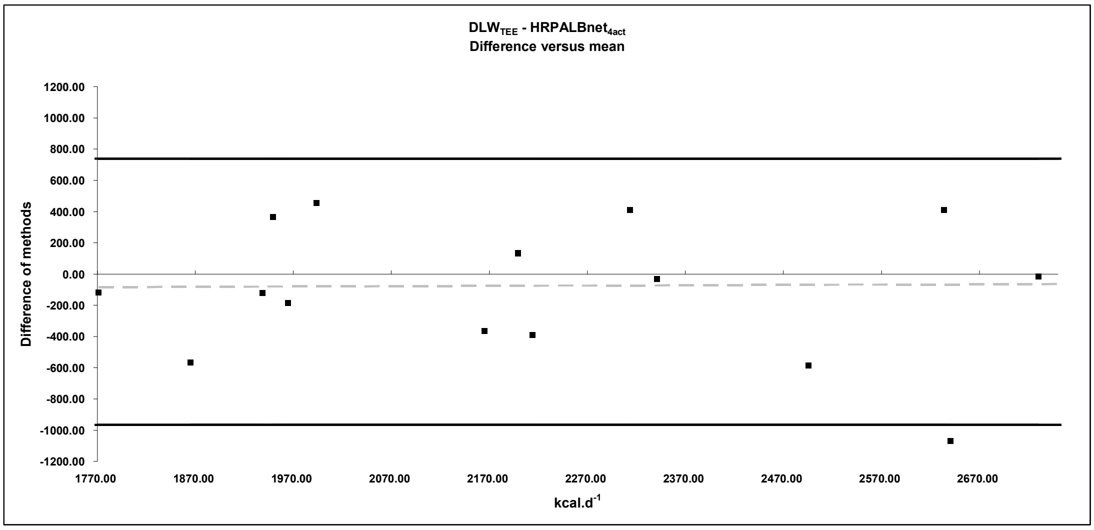


Figure 4.20 - DLW$_{TEE}$ - HRPALBnet$_{4act}$

Mean difference: -113.1 95%CI: -329.4 to 103.2 SEE: 102.5



Using data from **AH$_{branched}$,** **HRPAnet$_{RMR}$**, **HRPAnet$_{4act}$** and **HRPALBnet$_{4act}$** comparisons between weekdays and weekends were performed and there was a statistically significant difference ($p<0.05$) with all techniques indicating higher energy expenditure during weekdays.

Having verified this difference between weekdays and weekends we only performed analyses for weekdays for **AH$_{branched}$,** **HRPAnet$_{RMR}$**, **HRPAnet$_{4act}$** and **HRPALBnet$_{4act}$**.

Figure 4.21 - DLW$_{TEE}$ X **AH$_{\mathbf{branched\ weekdays}}$**

Mean difference: -105.0 95%CI: -272.3 to 62.1 SEE: 79.3

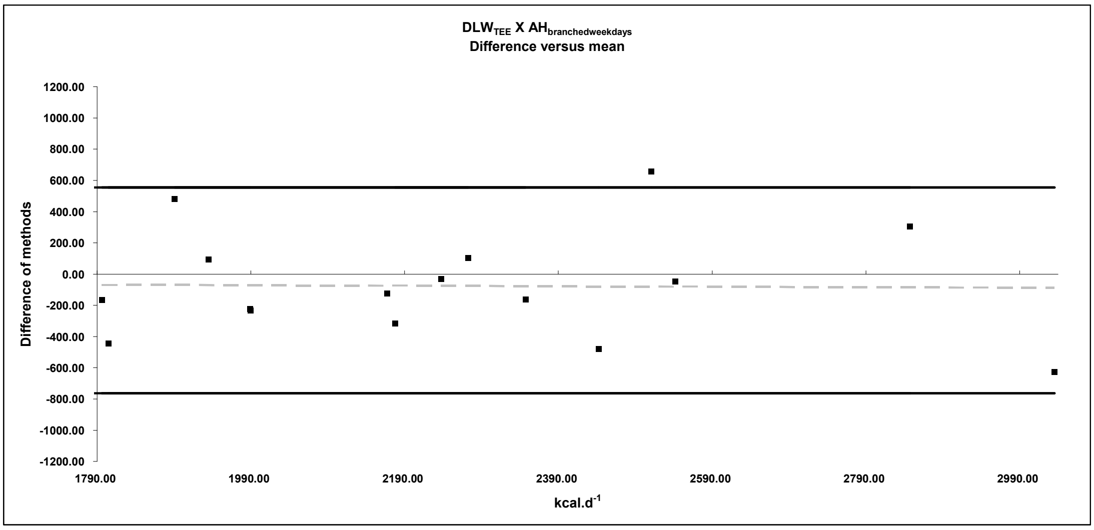


Figure 4.22 - DLW$_{TEE}$ X HRPAnet$_{RMRweekdays}$

Mean difference: -90.4 95%CI: -301.3 to 120.6 SEE: 100.0

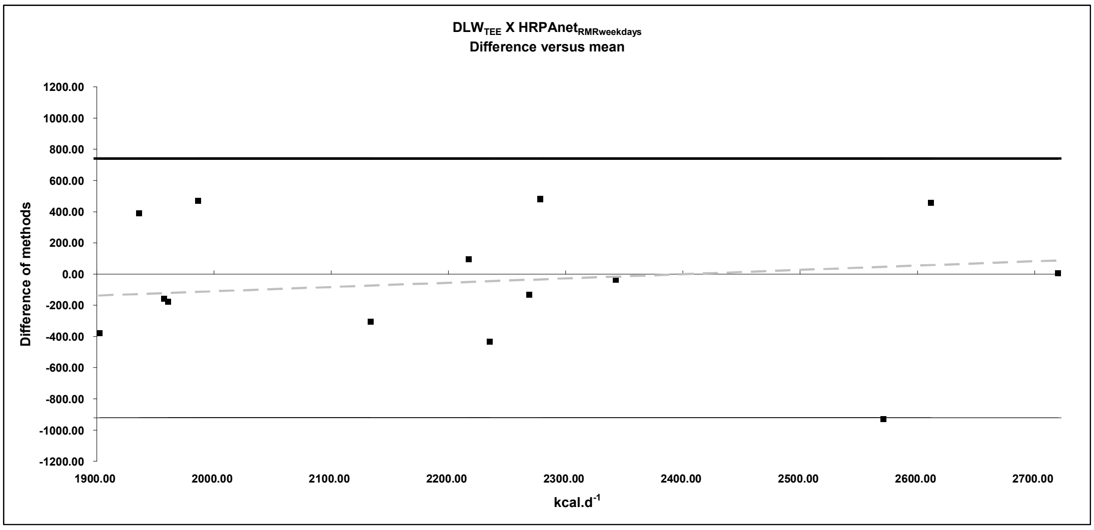


Figure 4.23 - DLW$_{TEE}$ X HRPAnet$_{4actweekdays}$

Mean difference: -133.0 95%CI: -345.0 to 78.9 SEE: 100.4

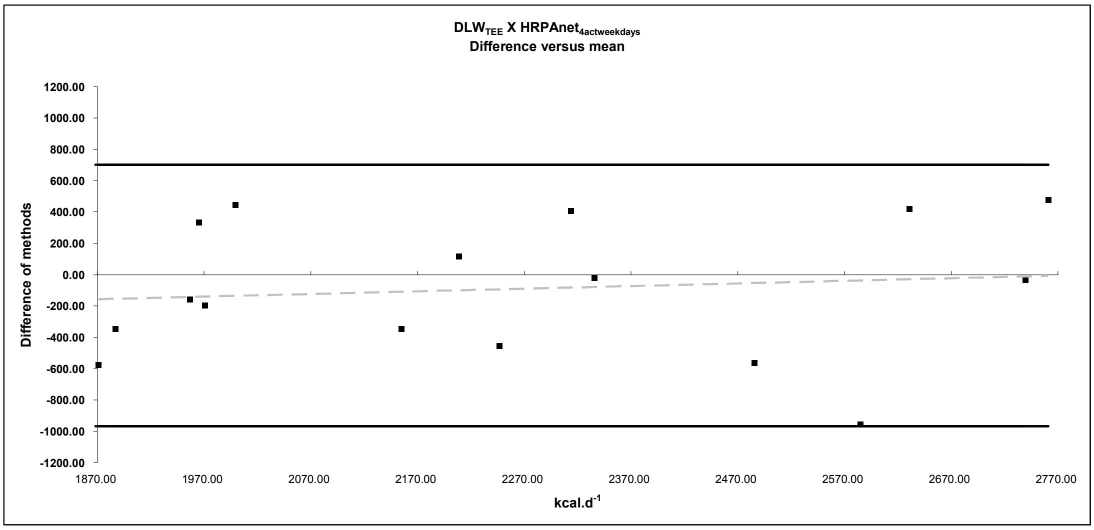
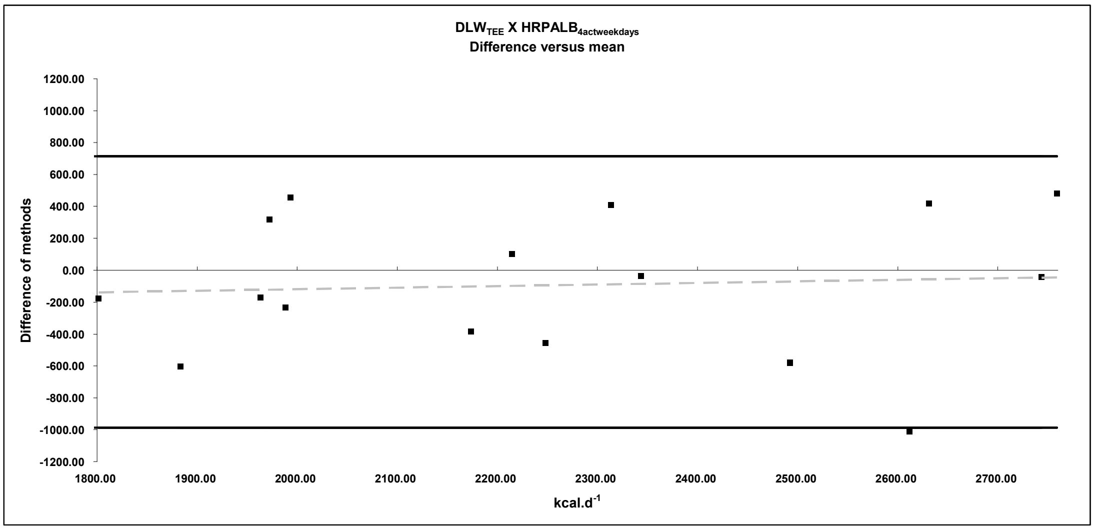


Figure 4.24 - DLW$_{TEE}$ X HRPALB$_{4actweekdays}$

Mean difference: -136.2 95%CI: -352.0 to 79.7 SEE: 102.3



Group equations

Multivariate relationships: Two models of multiple regression analyses were used. In the first regression model, stepwise multiple regression analysis was used to determine which of the independent variables contributed to the variation in AEE. Independent variables in this model included HR_{bpm}, AH_{cpm}, $ABankle_{cpm}$, $ABwaist_{cpm}$, $weight_{kg}$, $height_{m}$, body $fat_{\%}$, fat free $mass_{kg}$, and age_{years}. For the second regression model, a forced (enter) method was used including only $ABankle_{cpm}$ and $ABwaist_{cpm}$. AEE prediction equations were developed. To test the validity of these equations, the degree of agreement between predicted values and AEE measured using the DLW technique were tested using Bland and Altman (1986) analyses.

We subsequently used 2 equations from the first regression model. In the first equation, AEE was significantly influenced by HR_{bpm}, AH_{cpm}, $weight_{kg}$ and $age_{years.}$ In this model age (R^2= 0.02) showed the lower individual contribution to the total adjusted R^2. Considering this lower age contribution we used the second equation where AEE was significantly influenced by HR_{bpm}, AH_{cpm} and $weight_{kg}$ with a small reduction in the total adjusted R^2.

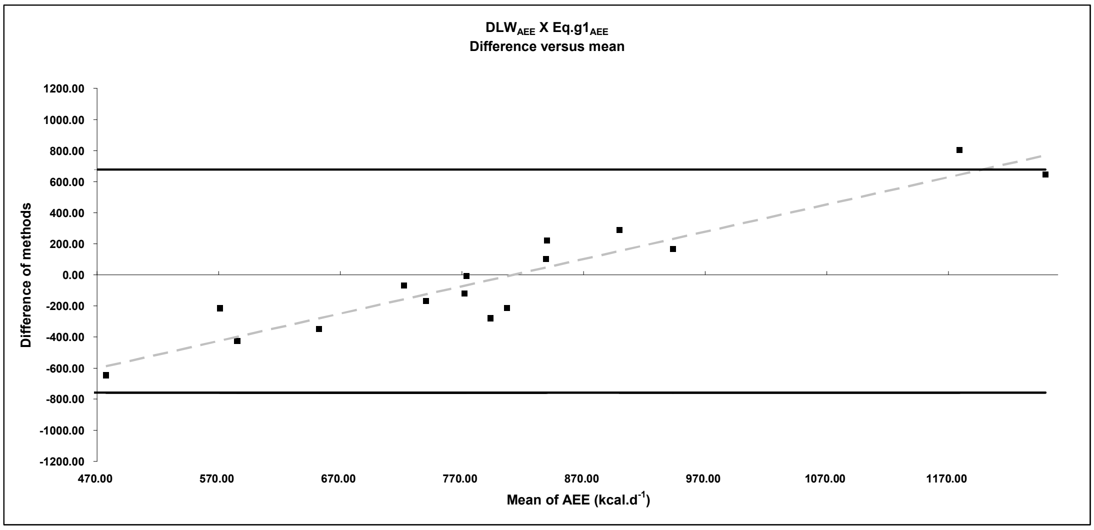
From this first model, general equations 1 and 2 as well as plots of the mean against the difference between DLW_{AEE} and equation $predicted_{AEE}$ are shown below:

General equation 1: $kcal.kg^{-1}.min^{-1}$

$$\mathbf{(-5.483+(HR_{bpm}*0.19)+(weight_{kg}*0.73)+(AH_{cpm}*0.001)+(age_{years}*0.26))/weight_{kg}}$$

Figure 4.25 - DLW$_{AEE}$ X Eq.g1$_{AEE}$ (kcal.d^{-1})

Mean difference: -40.4 95%CI: -222.4 to 141.6; SEE: 86.3

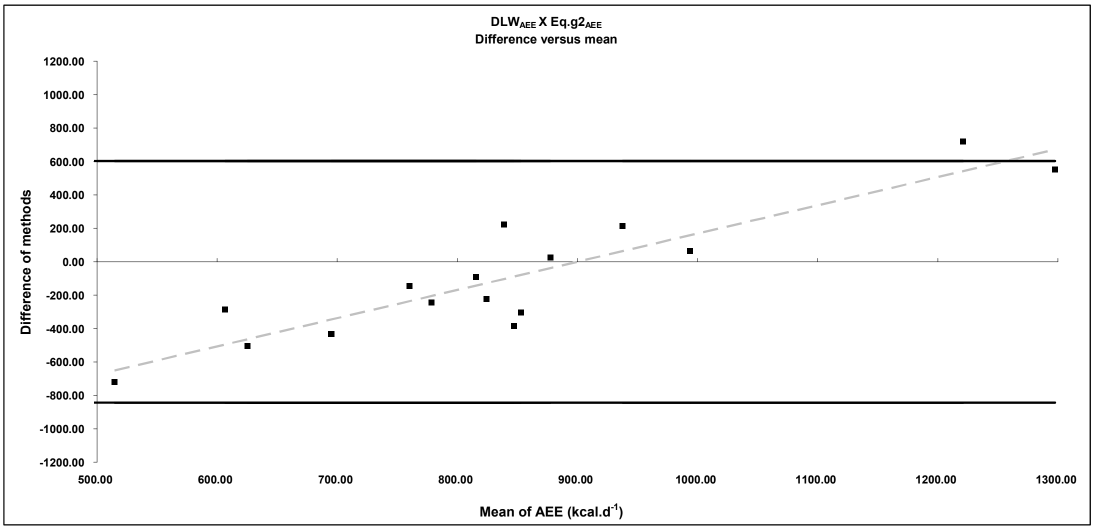


General equation 2: kcal.kg^{-1}.min^{-1}

$$\mathbf{(-3.179+(HR_{bpm}*0.22)+(weight_{kg}*0.78)+(AH_{cpm}*0.001))/weight_{kg}}$$

Figure 4.26 - DLW$_{AEE}$ X Eq.g2$_{AEE}$ (kcal.d^{-1})

Mean difference: -119.7; 95%CI: -303.0 to 63.7; SEE: 86.9



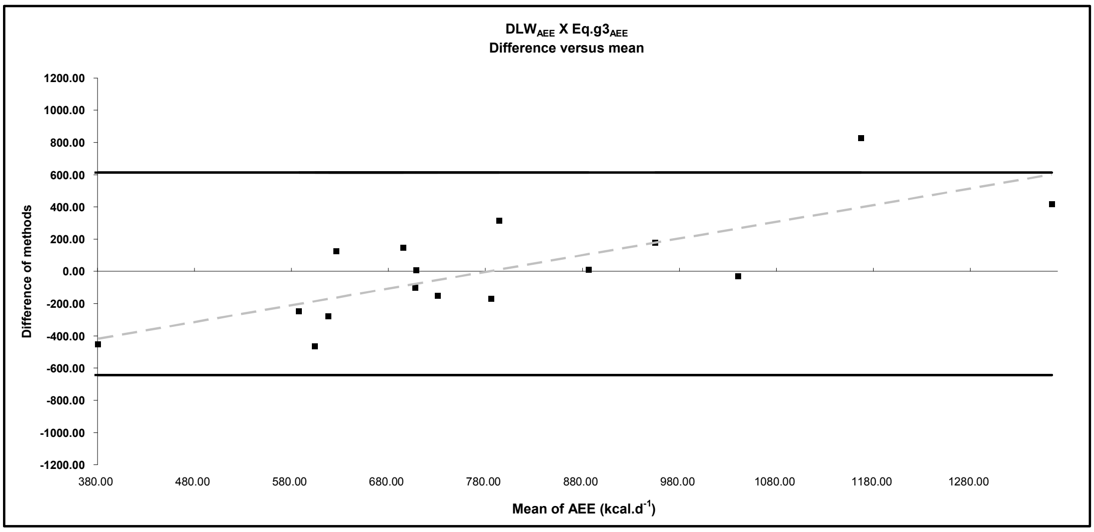
In the second model, AEE was significantly influenced by the two independent variables used, $\text{waist}_{\text{cpm}}$ and $\text{ankle}_{\text{cpm}}$ showing a total adjusted R^2 of 0.58. From this model, general equation 3 and plots of the mean against the difference between DLW_{AEE} and equation $\text{predicted}_{\text{AEE}}$ are detailed below:

General equation 3: $\text{kcal.kg}^{-1}.\text{min}^{-1}$

$$(0.747+(\text{Ankle}_{\text{cpm}}*0.002)+(\text{waist}_{\text{cpm}}*0.001)$$

Figure 4.27 - DLW_{AEE} X $\text{Eq.g3}_{\text{AEE}}$ (kcal.d^{-1})

Mean difference: -14.7; 95%CI: -174.0 to 144.6; SEE: 75.5



Using AEE from each regression (Eq.1, 2 and 3) plus measured RMR we subsequently estimated TEE. Plots of the mean against the difference between DLW_{TEE} and equation $\text{predicted}_{\text{TEE}}$ are shown below:

Figure 4.28 - DLW$_{TEE}$ X Eq.g1$_{TEE}$ (kcal.d^{-1})

Mean difference: 89.5; 95%CI: -109.2 to 288.2; SEE: 94.2

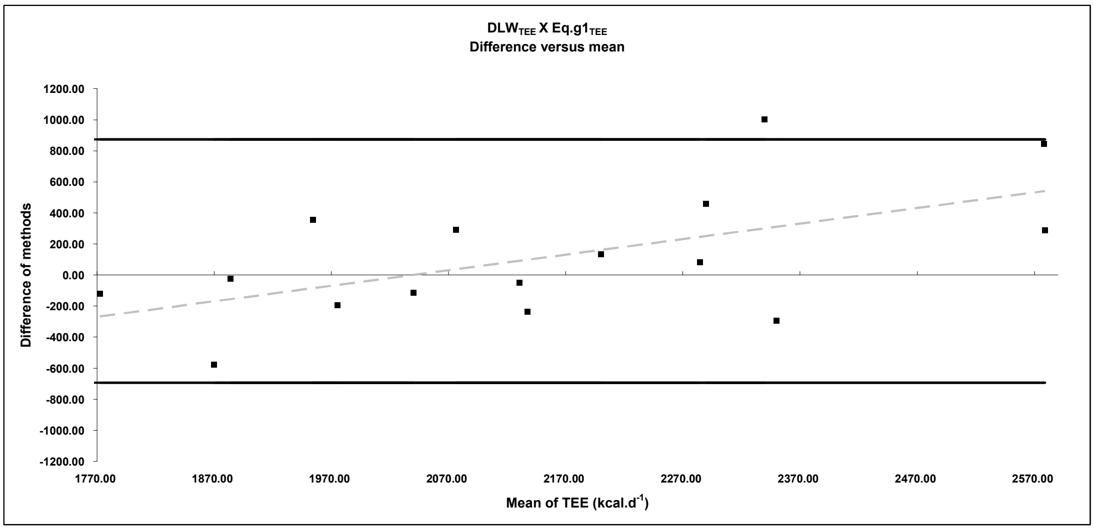


Figure 4.29 - DLW$_{TEE}$ X Eq.g2$_{TEE}$ (kcal.d^{-1})

Mean difference: 1.38; 95%CI: -198.9 to 201.6; SEE: 94.9

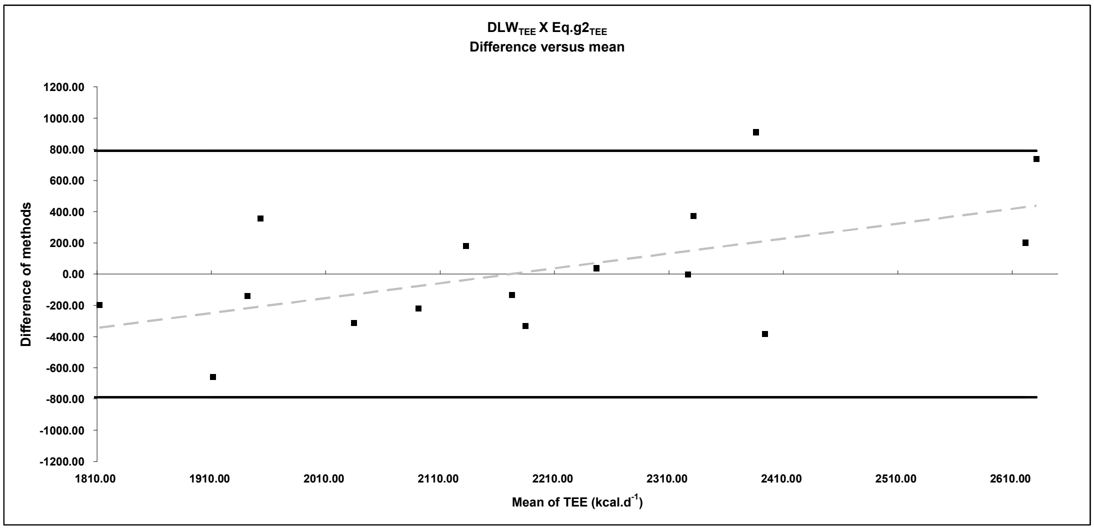
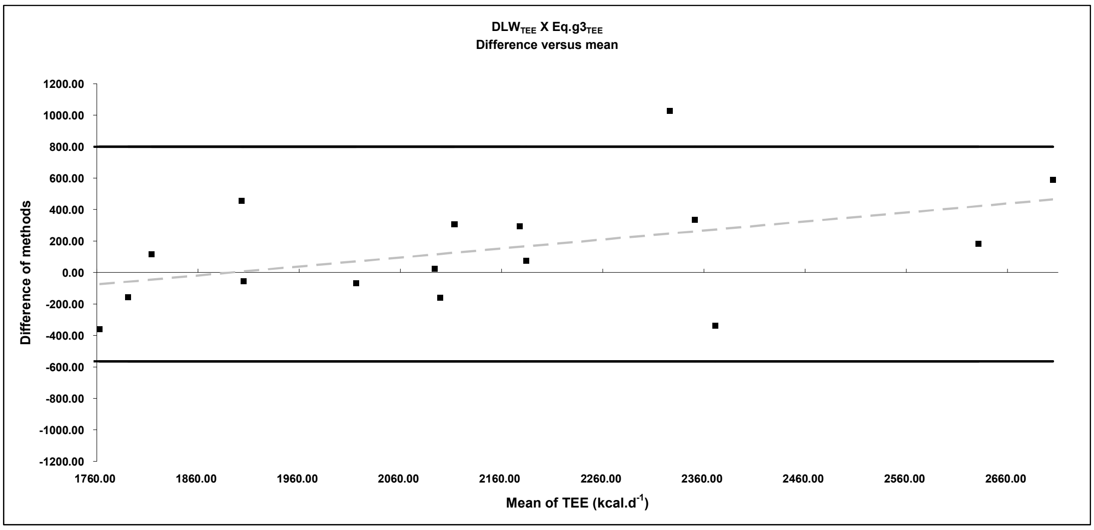


Figure 4.30 - DLW_{TEE} X $Eq.g3_{TEE}$ ($kcal.d^{-1}$)

Mean difference: 118.0; 95%CI: -55.0 to 291.0; SEE: 82.0



Cross-validation

In this model, AEE was significantly influenced by HR_{bpm} ($r = 0.74$; $p<0.001$), the independent variable used, showing a total adjusted R^2 of 0.54.

Group equation 4 ($Eq.4_{AEE}$) as well as plots of the mean against the difference between AEE measured the present study ($Study_{AEEmeas}$) and $Eq.4_{AEE}$ predictions are outlined below:

$Eq.4_{AEE}$: $kcal.kg^{-1}.min^{-1}$

$$(-1.134+(HR_{bpm}*0.032)$$

$R = 0.74$; $R^2 = 0.54$; adjusted $R^2 = 0.54$; SEE = 0.54

The cross-validation analyses showed no significant differences between predicted $Eq.4_{AEE}$ and $Study_{AEEmeas}$ (Mean difference: 1.8; 95%CI: -1.1 to 4.7; SEE: 1.4)

Pairwise comparisons between predicted TEE in free-living conditions using Eq.4$_{AEE}$ against DLW measured TEE were performed. The same comparison was performed regarding AEE.

Figure 4.31 - DLW$_{TEE}$ X Eq.4$_{TEE}$ (kcal.d^{-1})

Mean difference: -14.2; 95%CI: -209.2 to 180.8; SEE: 92.4

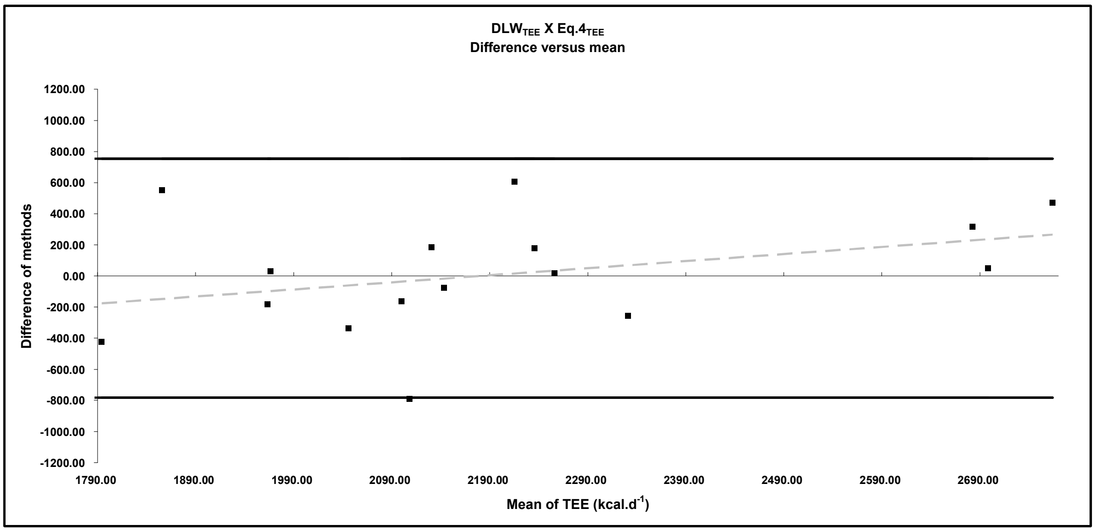
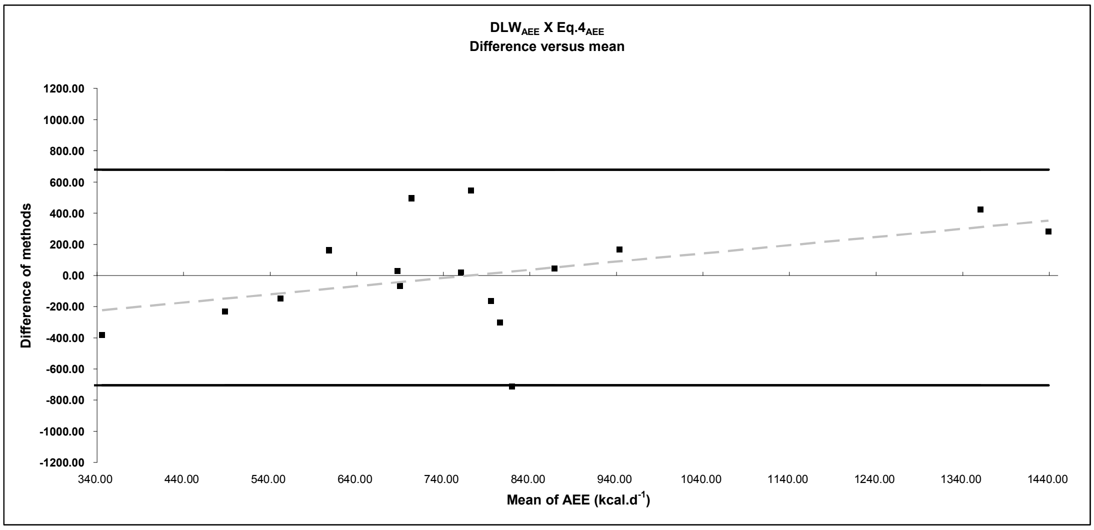


Figure 4.32 - DLW$_{AEE}$ X Eq.4$_{AEE}$ (kcal.d^{-1})

Mean difference: -12.8; 95%CI: -188.2 to 162.7; SEE: 83.2



Accelerometers

The mean total hours of monitoring per participant were 72.5±2.2 hours, or 12.1±1.4 hours of monitoring per day. The coefficient of variation (CV) of accelerometry within- and between participants using mean daily physical activity (MDPA) (total counts per minute / number of minutes) considering all days, weekdays and weekends are exhibited in Tables 4.8 and 4.9, respectively.

Table 4.8 – Accelerometer within-participant CV -
All days, weekdays and weekends

CV	AH	AB ankle	AB waist	AB wrist
All days				
Mean	32	35	41	27
Range	(14 to 73)	(10 to 64)	(18 to 88)	(10 to 67)
Weekdays				
Mean	28	32	38	25
Range	(11 to 73)	(11 to 73)	(12 to 88)	(4 to 59)
Weekends				
Mean	16	20	23	21
Range	(3 to 33)	(0 to 40)	(1 to 60)	(7 to 61)

Table 4.9 – Accelerometer between-participant CV –
All days, weekdays and weekends

CV	AH	AB ankle	AB waist	AB wrist
All days	38	44	64	23
Weekdays	38	42	62	22
Weekends	44	49	76	32

Intra-class correlation (ICC) between days for accelerometer MDPA considering all days, weekdays and weekends are displayed in Table 4.10 and for school days in different time windows (Blocks) in Table 4.11.

Table 4.10 – Accelerometer ICC - All days, weekdays and weekends

Accelerometer	All days	Weekdays	Weekends
AH	77	45	90
	(48 to 93)	(19 to 80)	(70 to 97)
AB ankle	76	56	90
	(44 to 93)	(16 to 85)	(66 to 97)
AB waist	45	34	19
	(25 to 83)	(36 to 74)	(2 to 78)
AB wrist	49	55	76
	(16 to 84)	(1 to 82)	(15 to 93)

Table 4.11 – Accelerometer ICC - School days by blocks

Accelerometer	Block 1	Block 2	Block 3	2 h after Sch
AH	70	83	73	74
	(10 to 94)	(48 to 97)	(20 to 95)	(22 to 95)
AB ankle	53	73	76	76
	(61 to 80)	(47 to 89)	(52 to 90)	(52 to 90)
AB waist	64	60	67	69
	(28 to 85	(20 to 85)	(35 to 86)	(38 to 87)
AB wrist	72	74	64	61
	(45 to 88)	(49 to 89)	(28 to 85)	(24 to 84)

Data analyses from all four accelerometers used to compare Saturday and Sunday showed no significant difference between weekend days for any accelerometer.

Our study design allowed us to verify the relationship between MDPA data from accelerometers at each body location with AEE and TEE from DLW. Logarithms were calculated because of the heteroscedasticity of counts at increasing activity levels as well as for between-participant energy expenditure.

AEE - *Pearson* correlations showed that MDPA was significantly ($p<0.01$) associated with DLW_{AEE} ($r = 0.27$ and 0.35 for AH (chest) and waist, respectively). Partial correlation adjusted by weight, height and body fat between DLW_{AEE} and MDPA were significantly ($p<0.01$) related (partial r: 0.22, 0.42 and 0.36, respectively for AH (chest), waist and wrist).

TEE – No association was verified between MDPA in any body location and $DLW_{TEE.}$ However, after adjusting for weight, height and body fat, MDPA were significantly ($p<0.01$) related to DLW_{TEE} (partial r were: 0.23; 0.39 and 0.50, respectively for AH (chest), waist and wrist).

Analyses by different time windows showed statistically significant differences ($p<0.05$) between block 2 and block 1, 3 and 4 for ankle, waist, wrist and chest mounted accelerometers, as well as for the sum of all three Actiband accelerometers. Block 1 as the less active, and block 2 as the most active among all verified time windows during school days was evidenced. However, no statistically significant difference was found between blocks 1, 3 and 4.

Pearson correlations between TEE from DLW and MBPA from accelerometers at each body location in each time window showed statistically significant associations for block 3 (ankle: r=0.47, p=0.001; waist: r= 0.38, p=0.002; Chest: r=0.49, p=0.001) and Block 4 (ankle: r=0.46, p=0.001; waist: r= 0.29, p=0.02; Chest: r=0.43, p=0.001), for both after school periods.

Partial correlation among DLW and MBPA adjusted by weight, height and body fat showed statiscally significant results for block 1 at waist (r=0.29, p=0.03); block 3 at ankle (r=0.33, p=0.01); wrist (r=0.27, p=0.04) and chest (r=0.29, p=0.03); and block 4 at ankle (r=0.28, p=0.04).

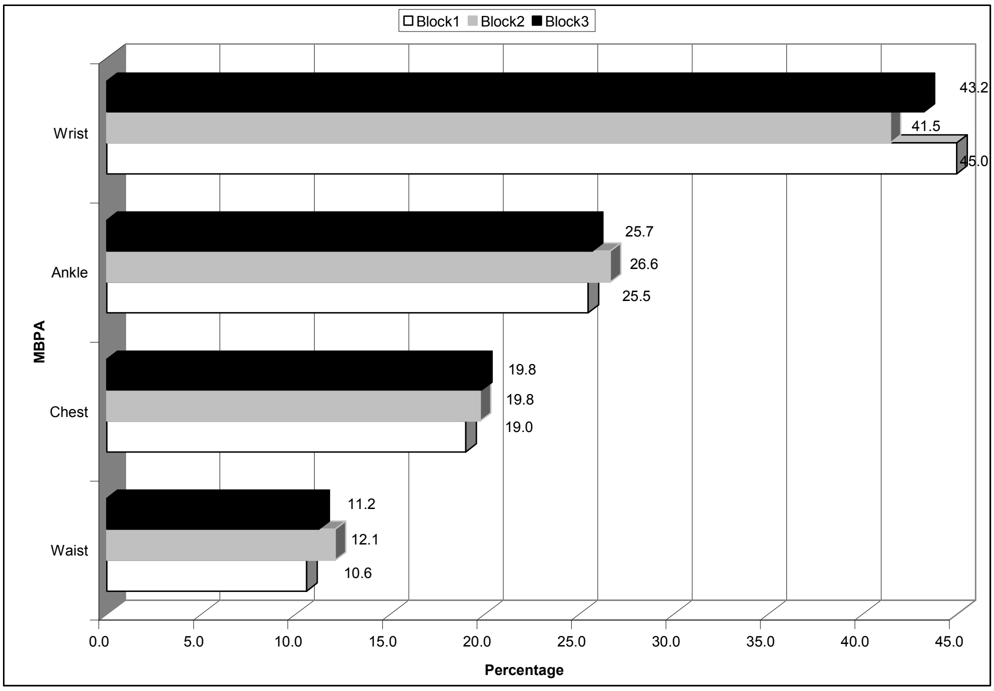
The relative between-block participation in the MDPA (here expressed as the sum of all 3 MBPA) can be seen in Table 4.12.

Table 4.12 – Relative between-block participation in MDPA

Blocks	Ankle	Waist	Wrist	Chest
1	28.9	27.4	30.7	28.6
2	38.0	39.5	35.7	37.5
3	33.1	33.1	33.6	33.9

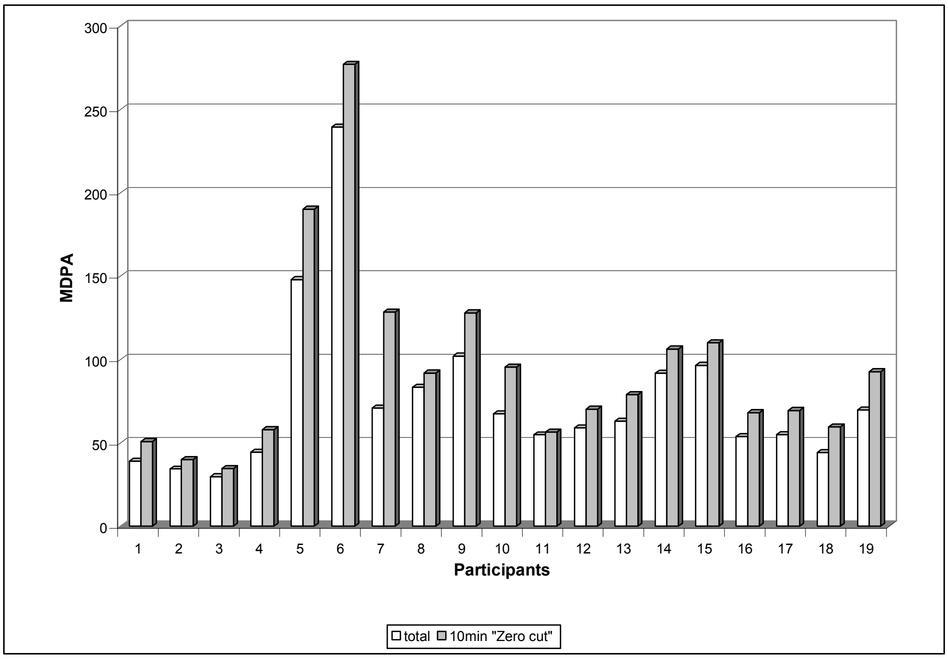
The relative MDPA participation of each body segment for the total block count is displayed at the Figure 4.33 below.

Figure 4.33 – Relative MDPA participation by body segment



Paired T-test showed a significant difference between the two data cleaning procedures used (p<0.001). Mean differences between procedures showed a 20% overestimation in MDPA using the habitual "Zero cut" procedure when all days were analysed (Figure 4.34) or only weekdays, and a 23% overestimation at weekends.

Figure 4.34 – Individual difference between cleaning procedures



Heart Rate

HR technique coefficient of variation (CV) within and between participants using estimated TEE from FlexHR$_{UBLB}$, AH$_{branched}$, HRPAnet$_{RMR}$, HRPAnet$_{4act}$ and HRPALBnet$_{4act}$ considering all days, weekdays and weekends are exhibited in Tables 4.13 and 4.14, respectively.

Table 4.13 – HR TEE within-participant CV - all days, weekdays and weekends

CV	FlexHR$_{UBLB}$	AH$_{branched}$	HRPAnet$_{RMR}$	HRPAnet$_{4act}$	HRPALBnet$_{4act}$
All days					
Mean	14	11	8	8	8
Range	(6 to 29)	(5 to 19)	(3 to 15)	(2 to 16)	(2 to 23)
Weekdays					
Mean	13	9	8	7	8
Range	(5 to 30)	(2 to 22)	(2 to 15)	(2 to 16)	(2 to 26)
Weekend					
Mean	13	9	6	6	6
Range	(3 to 38)	(3 to 30)	(1 to 21)	(1 to 20)	(1 to 21)

Table 4.14 – HR TEE between-participant CV - all days, weekdays and weekends

CV	$\text{FlexHR}_{\text{UBLB}}$	$\text{AH}_{\text{branched}}$	$\text{HRPAnet}_{\text{RMR}}$	$\text{HRPAnet}_{\text{4act}}$	$\text{HRPALBnet}_{\text{4act}}$
All days	20	18	21	20	21
Weekdays	20	19	21	21	21
Weekends	25	19	21	21	22

Intra-class correlation (ICC) between days for HR TEE from $\text{FlexHR}_{\text{UBLB}}$, $\text{AH}_{\text{branched}}$, $\text{HRPAnet}_{\text{RMR}}$, $\text{HRPAnet}_{\text{4act}}$ and $\text{HRPALBnet}_{\text{4act}}$ considering all days, weekdays and weekends are shown in Table 4.15.

Table 4.15 – HR TEE ICC - All days, weekdays and weekends

Technique	All days	Weekdays	Weekends
$\text{FlexHR}_{\text{UBLB}}$	94	88	82
	(86 to 98)	(74 to 96)	(42 to 95)
$\text{AH}_{\text{branched}}$	96	96	82
	(92 to 99)	(90 to 98)	(40 to 94)
$\text{HRPAnet}_{\text{RMR}}$	98	95	90
	(94 to 99)	(89 to 98)	(66 to 97)
$\text{HRPAnet}_{\text{4act}}$	97	95	92
	(93 to 98)	(88 to 98)	(75 to 98)
$\text{HRPALBnet}_{\text{4act}}$	96	94	86
	(91 to 99)	(86 to 98)	(55 to 96)

Our study design also allowed us to verify the relationship between data using HR techniques with AEE and TEE from DLW. Before each analysis, an assessment of data normality was undertaken.

TEE - correlation among DLW_{TEE} and HR techniques adjusted by body fat were 0.65, 0.64, 0.64, 0.62 and 0.44, respectively for $\text{AH}_{\text{branched}}$, $\text{HRPAnet}_{\text{RMR}}$, $\text{HRPAnet}_{\text{4act}}$, $\text{HRPALBnet}_{\text{4act}}$ and $\text{FlexHR}_{\text{UBLB}}$, all statistically significant ($p<0.01$). Interestingly, when adjusted by weight only, $\text{AH}_{\text{branched}}$ (0.30, $p<0.01$) and $\text{HRPAnet}_{\text{RMR}}$ (0.19, $p<0.05$) were significantly associated with DLW_{TEE}.

AEE - *Pearson* correlations showed statistically significant associations ($p<0.01$) among all analysed HR techniques with DLW_{AEE} ($r = 0.43, 0.39, 0.35, 0.35$ and 0.25, respectively for $AH_{branched}$, $HRPAnet_{RMR}$, $HRPAnet_{4act}$, $HRPALBnet_{4act}$ and $FlexHR_{UBLB}$. Again, when adjusted by weight, only $AH_{branched}$ (0.19, $p<0.05$) was associated with DLW_{AEE}.

An analysis by different time windows with the same methodology as used for accelerometers was also undertaken by applying the $HRPAnet_{RMR}$ technique to measure AEE. To allow a comparison among time windows with different duration (i.e.: block 4) the equation exhibited in section 4.2.2.4.1 was utilised for time adjustments.

Mean values and SEM for each time window, from block 1 to block 4 respectively, were 283±14; 322±30; 294±22 and 311±26. Repeated measures ANOVA showed statistically significant differences among block 1 and blocks 2, 3 and 4. Pearson correlation showed associations between AEE from DLW and block 3 ($r=0.24$, $p=0.04$) and block 4($r=0.32$, $p=0.009$) as well as partial correlation controlled by BMI showed association between DLW and block 3 ($r=0.26$, $p=0.03$) and block 4 ($r=0.33$, $p=0.008$).

4.2.4 Discussion

4.2.4.1 Accelerometers and speed detection

We are aware that not using the same absolute speed for all participants can reduce the speed sensitivity however despite this we strongly believe that this procedure was the most suitable for the present study. Speed determination based on self-selected speed and derived speeds that are representative of transportation speeds habitually used in free-living conditions are critical. It is important to highlight that all participant's treadmill activities were performed based on the individual's preferred speeds of locomotion. This opinion is shared by others (Tanaka, Tanaka, Kawahara, & Midorikawa, 2007) who agree that a self-selected pace more precisely reflects actual energy expenditure and accelerometer counts of children undertaking activities in the real world as children do not perform activities at predetermined speeds.

The Actiheart has been validated in children (Corder, Brage, Wareham, & Ekelund, 2005) and adults (Brage, Brage, Franks, Ekelund, & Wareham, 2005; Brage et al., 2007) and is highly linear with acceleration (r=0.99), regardless of movement frequency. Actiheart and Actiband movement sensors have the same technical characteristics as outlined in sections 4.2.2.3.1.1 and 4.2.2.3.1.2, respectively. Despite previous Actiheart validation studies, we included Actiheart laboratory data based on our self-selected speed design together with waist and ankle mounted Actibands. AH_{cpm} and $ABwaist_{cpm}$ showed a similar ability to detect changes in walking and running speeds as well as among all walking and running speeds analysed. These results are similar to those verified for the Tritac-R3D (Professional products, A division of Reining Int., Madison, WI) and Computer Science & Applications (CSA – model 7164, Shalimar, FL) accelerometers using the same 0.8 $km.h^{-1}$ speed increment (Leenders, Nelson, & Sherman, 2003).

However, $ABankle_{cpm}$ was able to differentiate all walking speeds up to V4 (> 0.8 $km.h^{-1}$ than SS), but was limited in the ability to differentiate running speeds (V5 – V6). A high-frequency component of the limb movements, the strongest influence of the gravitational component for limb accelerometer placement (Bouten, Sauren, Verduin, & Janssen, 1997), and the theoretical construct that acceleration increases in several dimensions with faster movements, suggests that accelerometers may misinterpret the greater body movement associated with reduced mechanical efficiency (King, Torres, Potter, Brooks, & Coleman, 2004) habitually verified at faster speeds. These influential characteristics may help to explain the verified limitation of $\text{ABankle}_{\text{cpm}}$ to differentiate running speeds. Despite this drawback, when utilised during 7-day free-living conditions $\text{ABankle}_{\text{cpm}}$ showed an acceptable ICC of 76% as well as being significantly associated with TEE from DLW at time windows after school. Also, when considering the relative MDPA contribution of each body segment, the ankle was the second most representative body location.

The output from an accelerometer is dependent on its position on the body as well as the inherent mechanical properties of the sensor. There is little evidence to suggest that one position is better than another (Welk, 2005). The most common position is the hip, but output may vary even with physical position of the device about the hip (Yngve, Nilsson, Sjostrom, & Ekelund, 2003). Despite recommendations to avoid accelerometer placement on the wrist or ankle, the use of multiple accelerometers has not been rigorously evaluated in children. Most importantly, the ability to measure arm or limb movements in conjunction with movements of the torso may be helpful in detecting the nonambulatory activities typically exhibited by the younger population (Trost, McIver, & Pate, 2005).

It is interesting to note that no calibration study to date has used sites other than the trunk to derive equations for interpreting accelerometer output (Ward, Evenson, Vaughn, Rodgers, & Troiano, 2005). Some authors have suggested that studies are needed to determine whether more than one accelerometer is required to measure the scope of children's physical activity (Chen & Bassett, 2005; Trost et al., 2005; Ward et al., 2005)

4.2.4.2 Total energy expenditure

The physical activity level of an individual is commonly described by the ratio of TEE to RMR (TEE/RMR) (WHO, 1985) and known as the physical activity level (PAL). Describing physical activity habits in terms of PAL is not entirely satisfactory because the increments in energy expenditure brought about by most physical activities are directly proportional to body weight, whereas RMR is proportional to body weight$^{0.75}$ (Brooks et al., 2004). Our data are in partial agreement with this premise showing significant ($p<0.01$) associations among RMR, weight ($r=0.79$) and weight$^{0.75}$ ($r=0.80$), as well as among TEE, weight ($r=0.77$) and weight$^{0.75}$ ($r=0.76$). However, as PAL is a widely used construct and provides a convenient notion of physical activity we had the option of using PAL to characterise our sample and to allow between-study comparisons (as well as comparisons with other physical activity indices used in this document). Considering the two most active categories, 73.6% of our sample was classified as adequately active, indicating that 26.4% were below the active cut-off. The overall sample mean for PAL was 1.86±0.43, results that are in agreement with a study performed in a US sample of similar age and anthropometric characteristics (DeLany, Bray, Harsha, & Volaufova, 2006) and with the normative DLW database from the chronicle of the Institute of Medicine physical activity recommendation (Brooks, Butte, Rand, Flatt, & Caballero, 2004), all classified as active based on PAL from DLW measurements.

Reference data for PAL as a function of age was proposed from analyses of 17 studies based on TEE measurement by DLW among children aged 3–16 years (Hoos et al., 2003a). Comparisons using data from the present study and the proposed equation showed a high association ($r = 0.98$), no statistically significant difference and the same standard error (SEM = 0.10). From the public health perspective where study designs using the DLW technique are not possible in large samples due to high cost of isotopes, the utilisation of equations to estimate PAL would be an interesting alternative for population surveys, at least as a screening tool if other objective techniques for physical activity measurement are not available.

Comparisons between measured TEE_{dlw} and all prediction techniques utilised showed that $\text{AH}_{\text{branched}}$ was one of the most adequate tools for TEE measurement in free-living conditions in children. The AH has been validated in mechanical settings (Brage et al., 2005) and during walking and running in controlled laboratory conditions in adults (Brage et al., 2005) and children (Corder et al., 2005) as well as in controlled field settings in adults (Crouter et al., 2007). However the present study is the first to generate results using the Actiheart in a ‘real world’ free-living context and validated against a gold standard method.

4.2.4.2.1 Actiheart - Branched model

It is important to note that among all techniques used in this first part of the present study only $AH_{branched}$ was based on a group equation. Each of the other techniques used were based on individualised regression which is theoretically more accurate. However, no significant differences between $AH_{branched}$ predicted and measured DLW TEE were verified, showing an SEE of 79 $kcal.d^{-1}$ or 3.6% of the mean group measured TEE. On the other hand, the mean difference was verified as 72 $kcal.d^{-1}$, representing a mean individual difference of -4.4% with a 95% CI ranging from -238 to 93.9, which represents -10.8 to 4.3%. These results suggest that $AH_{branched}$ which combines activity and the HR algorithm provides similar estimates of TEE in children under free-living conditions on both a group and an individual basis. Our results are in agreement with a recent study in a controlled field setting using Actiheart in adults (Crouter et al., 2007).

Since the conceptual basis and preliminary evaluation of a procedure using the simultaneous recording of heart rate and motion sensors was applied by Avons (1988), a series of studies using simultaneous HR and movement sensors have been conducted (Haskell et al., 1993; Moon & Butte, 1996; Luke et al., 1997; Treuth et al., 1998; Beghin, Budniok, Vaksman, Boussard-Delbecque, Michaud, Turck, & Gottrand, 2000; Strath et al., 2001; Strath et al., 2002; Brage et al., 2004; Plasqui & Westerterp, 2005; Johansson, Rossander-Hulthen, Slinde, & Ekblom, 2006). However, to perform measurements using HR and motion sensor simultaneously some drawbacks have been identified. These include an increased burden to young participants when using multiple devices for a long period of time, potentially reducing the compliance across the monitoring period, as well as difficulties for data reduction, processing and analysis by the researcher. The AH technology introduces the possibility of the simultaneous measurement of heart rate and movement counts from a unique small device reducing this double-burden. Another advantage of the AH is the built-in branched model combining accelerometry and heart rate originally proposed by Brage et al. (2004) designed as a framework to interpret simultaneous HR and accelerometry data in minute-by minute physical activity intensity.

The main drawback of the use of the Actiheart device for a long monitoring period such as 7 days in the present study is that skin irritation was experienced by some participants. Before the Actiheart can be used, the participant is fitted with ECG pads to which the Actiheart is clipped. Selection of the most appropriate pads is difficult due to significant variability in skin types. In the present study we experimented with all electrodes suggested by the Actiheart manufacturer, but in some participants it was a challenge to use the device for more than 3 days. Despite not being a serious cause for concern, the redness occasioned by the pads in some children could introduce a barrier to full compliance to the protocol by some parents. A useful suggestion may be for children with previously known allergic problems to experiment with 2 or 3 different pads for few hours before starting a formal monitoring period. Such a problem can be minimised during longer monitoring periods by applying hydrocortisone cream in case of any skin reaction.

Despite the above mentioned problem, the device was very well accepted by children because it is small, light, water-proof and able to be used underneath clothes. From the perspective of the researcher, advantages of the device are the absence of a button which could be tampered with by children, the use of a unique device to avoid time-synchronisation problems among accelerometer and HR when using each device separately. Further, there was low participant reactivity to the use of multiple devices and this, along with very well designed software allowed a range of analyses and data exportation to statistical packages.

4.2.4.2.2 *Net* values - Physical activity and heart rate

Among all individually-based techniques where the mean of the differences was not significantly different from zero, three were superior to the others, namely $\mathrm{HRPAnet}_{\mathrm{RMR}}$, $\mathrm{HRPAnet}_{\mathrm{4act}}$ and $\mathrm{HRPALBnet}_{\mathrm{4act}}$. Of these three techniques, $\mathrm{HRPAnet}_{\mathrm{RMR}}$ was only slightly more accurate than $\mathrm{HRPAnet}_{\mathrm{4act}}$ and $\mathrm{HRPALBnet}_{\mathrm{4act}}$, but as this technique is only adjusted by RMR with the other two heavily dependent on more complex laboratory calibration, results of the present study indicate that $\mathrm{HRPAnet}_{\mathrm{RMR}}$ is the best HR-based technique used. It is important to note that all three calibration equations are based on $\mathrm{HR}_{\mathrm{net}}$ [HR exercise minus sleep HR (SHR)] and $\mathrm{PA}_{\mathrm{net}}$ (measured PA exercise minus measured RMR) while three similar equations ($\mathrm{HRUBLB}_{\mathrm{RMR}}$, $\mathrm{HRUBLB}_{\mathrm{4act}}$ and $\mathrm{HRLB}_{\mathrm{4act}}$) were developed using *gross* HR and PA data.

This reduced inter-individual variance in regression equations relating heart rate and energy expenditure using *net* rather than *gross* measures was reported earlier (Andrews, 1971), and more recently by others who showed a significantly increased predictive value when using net HR (Luke et al., 1997).

Individual variation in gender, age, and training status have been shown to affect the HR-$\dot{V}O_2$ relationship (Davis & Convertino, 1975) however, in this study only females of similar age were measured. It has also been reported that the use of *net* heart rate allows comparison of activity between individuals of different fitness level (Freedson & Miller, 2000).

The explanation for the verified *net* and *gross* heart rate predictive differences lies in the wide between-subject variation in the resting heart rate, which is primarily dependent on fitness condition, whereas energy expenditure is primarily a function of body size. The low correlations at rest between heart rate and energy expenditure contribute to the between-subject variations in regression equations based on *gross* measures. However, the magnitude of the changes in heart rate and energy expenditure from their resting levels, *net* measures, are related similarly between-subjects.

It has also been suggested that *net* values can reduce inter-individual variability and remove the need for individual calibration as required for the Flex-HR method (Sarton-Miller et al., 2003). This presupposition was endorsed by results in the present study, not only regarding Flex-HR but also when considering the perspective of individual calibration from the least ($HRPAnet_{RMR}$) to the more complex protocol ($HRUBLB_{4act}$, $HRLB_{4act}$, $HRPAnet_{4act}$ and $HRPALBnet_{4act}$). Our results are directly aligned to and we share the same concerns expressed by Livingstone et al. (2000) regarding the inclusion of a wide range of different activities in the calibration procedure which have to be conducted with caution because the demands of a lengthy protocol may be intolerable for some children.

No significant differences between predicted $HRPAnet_{RMR}$ and measured TEE using the DLW technique were verified, with an SEE of 99 $kcal.d^{-1}$ which represented 4.5% of the mean group measured TEE. The mean difference was -67 $kcal.d^{-1}$ which represented a mean individual difference of -3.0% with a 95% CI ranging from -276.6 to 141.9, which represents -12.6 to 6.5%. No significant associations were observed between the mean and the difference for any of the plots, indicating that all predicted TEE values showed similar individual variation throughout the range of data. This suggests that $HRPAnet_{RMR}$ provides adequate estimates of TEE in children under free-living conditions. Our results are comparable to others who have used heart rate-based energy expenditure against DLW showing mean differences of -3.2% (Livingstone et al., 1992), 8.0% (Emons, Groenenboom, Westerterp, & Saris, 1992) and 0.2% (Maffeis, Pinelli, Zaffanello, Schena, Iacumin, & Schutz, 1995). However, Livingstone et al. (1992) and Maffeis et al. (1995) have used Flex-HR, while Emons et al. (1992) used linear regression as in the present study, but with a single day of HR monitoring.

4.2.4.2.3 Upper and lower body equations

Haskell et al. (1993) described an important concern about HR monitoring using the motion sensor data to determine when the person was active and establish if the activity was primarily leg exercise, arm exercise or a combination of the two, providing a more accurate estimate of energy EE than obtained from the use of only a regression determined for leg exercise. Others have applied this procedure in adults (Bot & Hollander, 2000; Strath et al., 2001; Strath et al., 2002; Strath et al., 2005) but in children we have found only one earlier study in which upper body activities were considered (Livingstone, Robson, & Totton, 2000), but in association with lower-body and resting activities in the same calibration equation and without verification against a more stringent method. The utilisation of combined arm-and-leg activity has been shown to be closely represented by the HR-$\dot{V}O_2$ relationship for leg activity (Haskell et al., 1993). A major strength of our design using $HRPAnet_{RMR}$ is a separate equation for the upper- and lower-body recognising movement predominance based on the assumption that the HR-$\dot{V}O_2$ relationship is affected by the relative size of the exercising muscle mass (Rowlands et al., 1997), with arm exercise eliciting a higher heart rate than leg exercise at the same $\dot{V}O_2$. The differentiation between upper and lower body work has been shown to refine EE estimates from HR in controlled field conditions (Strath et al., 2001) and free-living activities (Strath et al., 2002) in adults, but we are unaware of another study in children using this methodology in the same way. Therefore, a limited number of comparisons are possible with the literature.

4.2.4.2.4 Weekdays and weekends

Analyses from **$AH_{branched}$,** **$HRPAnet_{RMR}$**, **$HRPAnet_{4act}$** and **$HRPALBnet_{4act}$** showed statistically significant differences ($p<0.05$) between weekdays and weekends, with all techniques indicating higher energy expenditure during weekdays. However, comparisons considering only weekdays in the TEE estimates from these 4 techniques against TEE_{DLW}, despite no significant difference from zero, showed no evidence of prediction improvement, pointing to the need for the inclusion of weekends in TEE estimates as will be further discussed.

4.2.4.2.5 Group equations

We have applied 2 group equations derived from stepwise analyses in the first model. The first group equation from this model explains 87% of the variance in AEE. The individual contribution of each variable, from the largest to the smallest was $weight_{kg}$ ($r^2=0.24$), AH_{cpm} ($r^2=0.07$), HR_{bpm} ($r^2=0.03$), and age_{years} ($r^2=0.02$). The largest contribution coming from weight was expected, as in physical activities involving body displacement, EE is directly proportional to body weight. However, the lower contribution of AH_{cpm} and HR_{bpm} can be explained by the fact that when each variable is isolated the correlation values represent only the unique contribution of this variable, with any shared variance removed. In this case these two independent variables are strongly correlated ($r = 0.74$; $p<0.001$); therefore there is a lot of shared variance that is statistically removed when they are both included in the model. The lowest verified correlation with AEE in this model was from age ($r = 0.30$; $p<0.01$), despite being statistically significant. This finding is probably a function of the homogeneity verified by the restricted age interval of our sample. Considering this lower age contribution and the homogeneity of this variable in our sample we used a second group equation in this model. This second group equation explained 81% of the variance in AEE, with individual contribution from each of the remaining variables almost identical to the first equation: $weight_{kg}$ ($r^2=0.28$), AH_{cpm} ($r^2=0.06$) and HR_{bpm} ($r^2=0.03$).

In the second model, AEE was significantly ($p<0.001$) influenced by the two independent variables, $\text{waist}_{\text{cpm}}$ ($r^2=0.17$) and $\text{ankle}_{\text{cpm}}$ ($r^2=0.11$) showing a total adjusted R^2 of 0.58. Correlations with AEE were 0.60 and 0.55, respectively for $\text{waist}_{\text{cpm}}$ and $\text{ankle}_{\text{cpm}}$. These results are comparable to the findings of (Ekelund et al., 2001) in 9 year old children assessed by activity monitor and DLW where activity counts was significantly correlated to AEE ($r = 0.54$) and in a stepwise regression equation, was a unique predictor of AEE ($r^2=0.17$).

The three group equations from low-interference multi-system techniques (HR, activity counts) developed here were sufficiently accurate in the prediction of AEE and TEE at the group level for healthy children in free-living conditions. All showed acceptable SEE (75 to 87 kcal.d^{-1}), representing an error of 3.4% to 3.9% of the daily TEE. However, there were significant correlations ($p<0.05$) between the mean and the difference between methods, respectively from group equation 1 to 3 (TEE: $r=0.59$, $r = 0.57$, $r = 0.77$; AEE: $r=0.59$, $r = 0.57$, $r = 0.77$), indicating greater variation throughout the range of data, the wide limits of agreement introduce a limitation in the precision of individual estimates.

It should be considered that the accuracy of group equations in the prediction of AEE (or TEE) is limited because of the large variability within- and between-participants verified in the physical activity pattern in children (see Section 4.2.4.2.7 and 4.2.4.2.8). Provided that all limitations are recognised, and that errors in estimating AEE can be tolerated, group equation can be an interesting possibility mainly because individual calibration is not required.

4.2.4.2.6 Cross-validation

A laboratory-derived group equation using treadmill exercise from participant's data described in section 3.3 to predict AEE was applied to data from free-living conditions.

Reproducibility under laboratory conditions was verified for this equation by plotting the methods differences (Eq.4_{AEE} X $\text{Study}_{\text{AEEmeas}}$) against their mean, considering two standard deviations as the criterion for acceptable reproducibility.

This equation was applied to data from free-living conditions and pairwise comparisons between $Eq.4_{AEE}$ predicted and DLW_{AEE} were performed. The same comparison was performed regarding TEE. In this model AEE was significantly associated to HR_{bpm} ($r = 0.74$; $p<0.001$) explaining 54% of the total AEE variance. Prediction $Eq.4_{AEE}$ showed a mean difference of 13 $kcal.d^{-1}$ and SEE of 83 $kcal.d^{-1}$. Although individual estimates $Eq.4_{AEE}$ ranged from -24% to 21%, $Eq.4_{AEE}$ values lay within 2% of DLW_{AEE} estimates. Prediction $Eq.4_{TEE}$ individual estimates ranged from -9.5% to 8.2% and average values were within 6% of DLW_{TEE} estimates. The wide range showed by $Eq.4_{AEE}$ is in agreement with the observation that AEE is the most variable component of TEE (Goran, Carpenter, & Poehlman, 1993). However, when all components of EE were analysed together (TEE) this reported difference is diluted. It is important to stress that no significant associations were observed between the mean and the difference for any of the plots, indicating that values from prediction $Eq.4_{AEE}$ and $Eq.4_{TEE}$ showed similar individual variation throughout the range of data. These results suggest that $Eq.4_{AEE}$ provides acceptable estimates of TEE in children under free-living conditions at a group level. However individual estimates have to be carefully considered.

Some authors have argued that group calibration curves may increase the error of estimating EE and that it is specific to the activity performed and may not accurately reflect the EE of field activities (Rowlands et al., 1997). It should be added that the regression approach depends strongly on how well the method controls for inter-individual variability (Sarton-Miller et al., 2003). We have previously shown (section 3.3) that oxygen uptake based on self-selected speeds and derived speeds are reproducible in children. These speeds are habitually used in daily life, albeit not in all, but for the largest part of the day. Others are in agreement with this contention of Brooks et al. (2004) that walking is the most significant physical activity for most people and refer to how speed of locomotion and body mass affect the rate of EE, using this activity as a reference because the energy cost of transport is approximately constant for walking speeds in the range of 3 – 6 $km.h^{-1}$. It is likely that self-selected walking speeds more precisely reflect actual EE of children completing activities in the real world (Tanaka et al., 2007). Our design, supported by these arguments may help to explain, at least partially, the findings verified in the present study.

Some authors have speculated that prediction equations will probably never be as accurate as indirect calorimetry (Hilloskorpi, Fogelholm, Laukkanen, Pasanen, Oja, Manttari, & Natri, 1999) or DLW (Spurr, Prentice, Murgatroyd, Goldberg, Reina, & Christman, 1988b) in measuring individual EE. However, studies are in agreement that prediction equations can provide a reasonable estimate of EE even in small groups. HR monitoring does meet many of the criteria for evaluating free-living patterns of TEE and physical activity, and we cannot expect that a single method could address all the issues raised in this type of evaluation (Livingstone, 1997). Furthermore, the heart rate method can provide information on the pattern of daily activity not provided by the DLW technique.

4.2.4.2.7 Accelerometers

Within-participant CV for accelerometers MDPA showed a wide range of variability (range: 10% to 88%) in all accelerometers used. The largest variability verified for all days analysed was 27%, 32%, 35% and 41%, respectively, for AB_{wrist}, AH, AB_{ankle} and AB_{waist}. When only weekdays were analysed, a small reduction was verified in this variability, but differences among body location remained the same, with the largest CV verified at AB_{waist} in both analyses. Surprisingly, analyses of weekends not only showed lower CV for all accelerometers but also comparable values. Perhaps the reduced physical activity levels verified at weekends and consistently repeated on Saturdays and Sundays is a possible explanation for these findings, as will be further discussed later in this section.

Between-participant CV showed no difference between all days and weekdays, showing AB_{waist} with the largest CV in both, as previously shown in within-participant CV analyses. However, variability between-partcipants increased greatly at weekends, with the largest variability again verified at AB_{waist}.

Variability among individuals wearing the same waist accelerometer and completing the same absolute walking workload on a treadmill showed variability from 16% to 31% (McMurray et al., 2004; Welk, Schaben, & Morrow, 2004). It should be remembered that walking is a locomotor activity with highly consistent acceleration counts across time (Crouter, Clowers, & Bassett, 2006), while monitoring of free-living activities is expected to result in greater intra- and inter-day variability. These large CVs in a strictly controlled setting can not be readily compared to the free-living setting. Similarly, comparability among outputs from different devices is not adequate but can be used to illustrate the need for caution when using counts to establish cut-offs or to calibrate these counts with energy expenditure to give biological meaning to the output (Freedson et al., 2005)

Reliability analyses of data from all days of free-living monitoring was more reliable than only using weekdays for all different body locations of accelerometers, with AH (77%) and AB_{ankle} (76%) showing highest reliability. Lowest reliability was shown by AB_{wrist} (49%) and AB_{waist} (45%). Surprisingly, analyses of weekends showed increased ICC for all accelerometers, except for $AB_{waist.}$ As for comments regarding CV analyses, it may be that the reduced levels of physical activity, the most variable component than the sedentary behaviour, verified at weekends have inflated the ICC.

Our ICC results for monitoring all days of the week are in agreement with others who have conducted studies under free-living conditions (Nichols, Morgan, Sarkin, Sallis, & Calfas, 1999; Trost, Pate, Freedson, Sallis, & Taylor, 2000) and laboratory-based settings (Trost et al., 1998; Welk et al., 2004). (Penpraze et al., 2006) showed the number of days as more important to reliability than the number of hours monitored, but a marked ICC decrease if the monitoring period extended to 11 or more hours per day, irrespective of the number of days. There was little difference whether or not a weekend day was included. However, (Trost et al., 2000) showed that the transition from higher to lower physical activity level on weekends occurred earlier among girls than boys, suggesting from a methodological stand point the need to include weekend days when employing objective measures of physical activity in children and adolescents, as verified in the present study.

Penpraze et al. (2006) also suggested that short monitoring periods are appropriate for young children if the number of days is increased. However, (Trost et al., 2000) with a sample of similar age as in this study indicated that it was necessary to monitor for an entire day or sample from multiple times of the day. Our results for

ICC among school day blocks for MDPA are in agreement with this suggestion. We observed a wide ICC range for all accelerometers used. Different results can be seen for each accelerometer with limb based accelerometers (AB_{wrist} and AB_{ankle}) showing larger variability among blocks than trunk mounted accelerometers (AH and AB_{waist}). These reliability differences among blocks could be interpreted as an indicative of the need to monitor multiple periods of the day, but for practical reasons regarding monitor placement and collection as well as for compliance issues, the practice of wearing an accelerometer continuously should be recommended (Rowlands, 2007).

A number of studies using objective measures of physical activity in children have documented differences between weekdays and weekends (Armstrong, Balding, Gentle, & Kirby, 1990; Trost et al., 2000; Penpraze et al., 2006; Anderssen, Cooper, Riddoch, Sardinha, Harro, Brage, & Andersen, 2007; Hesketh, Salmon, & Crawford, 2007; Walkley et al., 2007), with marked age and gender influence on this behaviour leading to measurement recommendations for both types of days (Trost et al., 2000) or at least one of them in objective evaluation of physical activity in children. Recently, Walkley et al. (2007) investigated weekend data considering mean moderate-to-vigorous physical activity (MVPA) and showed marked differences between Saturday and Sunday. This suggests that the current recommendation of including at least one weekend day as representative of the total weekend may be in need of reconsideration. We have analysed our data from all four accelerometers used and compared results from Saturday and Sunday. We found no significant between-weekend day difference for any accelerometer. Independent of the biological similarity among groups of the same age and gender, different settings in various cultures, socio-economic status inside the same culture and environmental conditions inevitably are influential variable determinants of some behaviour, as physical activity throughout specific days. These contradictory, but not surprising findings emphasise the need to assess both weekend days as suggested earlier by (Trost et al., 2000) and recently by two literature review updates about accelerometry, one in children (Rowlands, 2007) and another for general application (Corder, Brage, & Ekelund, 2007).

Analyses by different time-windows throughout the day showed block 2 (between 12:01pm and 15:30pm) as the most active and block 1 (8:31am to 12:00 midday) as the least active, however no statistically significant differences between blocks 1, 3 and 4 were verified. Significant associations between TEE_{DLW} and MBPA were verified in blocks 3 and 4, both after-school hours for AH, AB_{waist} and AB_{ankle}.

Partial correlation among TEE_{DLW} and MBPA adjusted by weight, height and body fat, showed significant association again in both after-school hour blocks for AH, AB_{wrist} and AB_{ankle}. Surprisingly, a significant association for the AB_{waist} accelerometer, the most widely used site for accelerometry-based studies, was verified only in block 1, the least active time window in our study. If only one accelerometer is used at the waist, as is the case in most studies, one could make an erroneous conjecture based on this association. The careful observation of our Pearson correlation and partial correlation adjusted for weight, height and body fat with TEE provides verification that these results are a good indication that after-school time windows are representative of children's activity level. A suggested approach is to focus assessment on key times when children are active as the after-school time has been proposed as a critical period that defines children's propensity for physical activity (Welk, Corbin, & Dale, 2000). However the 2 hr time window after school is not necessarily the best considering that significant associations with measured TEE adjusted by anthropometric variables occurred in three of the four accelerometers used during the large after school time window (3.5 hr). Additionally, this large after-school period also showed an increased association with measured TEE for all accelerometers used when compared with the short after-school time windows. The after-school time was the consistent period of discretionary activity for girls of this age, as previously suggested by others using activity records completed by parents and accelerometry data (O'Connor et al., 2003). However, the limitation of this study was the short period of days monitored which may not be representative of children's activity patterns. Recently, Hesketh et al. (2007) showed that approximately half of the MVPA time was achieved during school hours and higher MVPA was seen during the after-school period, namely from the end of school to 6 pm, higher than either the before school and evening periods. However, the present study is the first to show an association between TEE measured using criterion method (DLW) and EE in the after school time window from a representative 7 days of monitoring.

When considering the relative contribution of each body segment there was a consistent predominance of wrist and ankle over chest and waist in all time windows. It is interesting to note that the chest-mounted accelerometer had an almost two-fold greater relative participation than the waist-mounted device. The most widely used body location in accelerometer studies, the waist, showed the smallest relative contribution among body locations assessed. These findings have to be carefully considered because the most common procedure in studies to objectively measure physical activity in children is to attach the accelerometer close to the centre of mass of the body (i.e: waist, hip or low back) (Westerterp, 1999). The position of attachment to the body can influence accelerometer output, with some showing only minor improvement in the explained variance in the energy expenditure using multiple accelerometers (Swartz, Strath, Bassett, O'Brien, King, & Ainsworth, 2000; Kumahara, Tanaka, & Schutz, 2004). There are clear limits on what can be achieved in free-living monitoring using a single waist-mounted accelerometer (Mathie et al., 2004a) with these authors suggesting that a greater number of instruments provides more information that allows more accurate activity classifications. However, the recommendation to use a single accelerometer in children is based mainly considering cost, compliance and reduced participant burden. The number of studies using multiple accelerometers on different sites of the body in this population is relatively small. Despite references to the use of multiple accelerometers in children (Hoos et al., 2003b; Corder et al., 2007; Rowlands, 2007), only two studies have included a range of calibration activities habitually performed in children under free-living conditions with multiple accelerometers. The first study was performed in a room respiration calorimeter for 6 hours (Puyau, Adolph, Vohra, & Butte, 2002) and another used indirect calorimetry for 1.5 hr under controlled laboratory conditions (Heil, 2006). In both studies, AEE was accurately estimated. Free-living conditions monitored using multi-system accelerometry, as in the present study, independent of the translation of counts in any other indirectly-derived output as AEE or MET, are valuable. Such approaches provide a broad view regarding movement predominance in children as well as the extra measurement axes may prove valuable for pattern recognition and other more sophisticated modelling techniques (Corder et al. 2007).

The first applicability of the multi-system accelerometry used here was to analyse one of the most common data reduction procedures. Habitually, data reduction procedures use parental records as the reference to exclude periods of repeated zero counts or 10 minute blocks of continuous zero measures (Andersen et al., 2006). However, periods of repeated zeros where the parental record gave no information have also commonly been excluded in studies (Riddoch et al., 2004; Penpraze et al., 2006; Baquet et al., 2007). In each of these studies, only one accelerometer has been used and attached to the waist region. In our study using 4 accelerometers on different body locations, we were able to verify that some 'real zero counts' at specific locations meant 'no movement' at that part of the body however there were movement counts at another body location. We have analysed our MDPA data using the waist-mounted accelerometer as a reference to make further comparisons with others studies in two ways. First, we have considered the information in the "not worn time log" and checked this against accelerometers' CPM and those periods of repeated zero counts corresponding to the records were excluded. Secondly, we used the same procedure but this time we excluded all 10 minute blocks of continuous zero records too. In both procedures, if zero records were verified in all used four accelerometers, this time interval was excluded. All participants in each day showed 'real zero counts' periods when considering recordings from the waist accelerometer. The habitual data reduction procedure cutting all 10 min sessions of consecutive zero values is significantly different ($p<0.001$) from the data reduction procedure using parental records only, showing an overestimation of around 20% in MDPA. This observation was only possible by the use of multi-system accelerometry which enabled the avoidance of the misclassification of time the accelerometer was 'not worn'. We strongly believe that studies in which all continuous 10 min zero records during the day are cut without this period of time recorded as "not worn time log", and further checked against accelerometer output, result in a gross overestimation of children's physical activity levels. Despite the availability of sophisticated procedures to input missing accelerometer data (Catellier, Hannan, Murray, Addy, Conway, Yang, & Rice, 2005) researchers must be cautious in the indiscriminate use of this or other similar procedures. At least until the standardisation of data reduction for accelerometers is developed, and an accepted criterion identified, data reduction procedures should be fully described to facilitate meaningful comparisons between studies.

Significant associations were verified between DLW_{AEE} and MDPA from AH and AB_{waist} as well as partial correlation adjusted by weight, height and body fat for AH, AB_{waist} and AB_{wrist}. However no significant association between TEE_{DLW} and MDPA from any body mounted accelerometer was verified, but again partial correlation adjusted by weight, height and body fat were significant for AH, AB_{waist} and AB_{wrist}. Individual differences in body size influencing movement counts associated with physical activity has been reported previously (Welk, 2005) as well as the observation that activity monitors are better at detecting whole body weightbearing activities and relatively insensitive to non-weight bearing activities (Melanson & Freedson, 1995). Under free-living conditions, activity monitors ankle and wrist-mounted are probably far more likely to record motions not related to the activity being performed (e.g, fidgeting) as shown by Heil (2006) under laboratory-controlled conditions. This earlier study, using a multi-system accelerometry system at ankle, hip and wrist suggested that total AEE for children can be accurately predicted. Ours results under free-living conditions are aligned with these laboratory-derived conclusions.

Accelerometers are very good tools to objectively measure physical activity patterns. However, there are some concerns regarding the transformation of counts to express movement as energy expenditure in kilocalories or metabolic units. The perspective of the insurmountable calibration needs for an infinite number of possible movements in the free–living situation must be considered. It is not surprising that count transformation from specific accelerometer placement at one or more body locations is not representative of all energy consumed by the whole body throughout a day or across a number of days given the wide within- and between-participant variability reported in this study. More advanced technologies are emerging to analyse accelerometer output, but as observed by (Trost et al., 2005) the use of multiple accelerometers has not been rigorously evaluated previously in children.

4.2.4.2.8 Heart rate

It is important to highlight the difference in approach between this section (HR) and the previous section (accelerometers). All data described in this section are considering TEE and AEE predicted from HR-based equations previously described in section 4.2.2.4.1. In contrast, in the previous section, activity counts were not transformed to any other indicator, but rather, mean counts were analysed for specific time periods.

Within-participant CV for TEE predicted by HR-based techniques showed lowest variability using $\text{HRPAnet}_{\text{RMR}}$, $\text{HRPAnet}_{\text{4act}}$ and $\text{HRPALBnet}_{\text{4act}}$ without significant differences between them for all days and weekdays, but a slightly reduced CV for weekends. The $\text{FlexHR}_{\text{UBLB}}$ technique showed higher variability in all analyses, independent of the day combination (that is,all days, weekdays and weekends). The $\text{AH}_{\text{branched}}$ showed lower CV than $\text{FlexHR}_{\text{UBLB}}$, but a little higher than $\text{HRPAnet}_{\text{RMR}}$, $\text{HRPAnet}_{\text{4act}}$ and $\text{HRPALBnet}_{\text{4act}}$. The simultaneous use of HR and accelerometry in the $\text{AH}_{\text{branched}}$ technique should be considered in this analysis. Considering the average result for all days by $\text{HRPAnet}_{\text{RMR,}}$ $\text{HRPAnet}_{\text{4act}}$ and $\text{HRPALBnet}_{\text{4act}}$ (8%; range: 3 – 15%) ours results under free-living conditions are similar to the mean CV of 10% earlier verified by (Livingstone et al., 1992) using DLW and 3 days of HR monitoring. Results are somewhat smaller than those verified in adults (mean: 16.6%; range: 6.8 – 21.5%) in 9 HR monitoring days (Davidson, McNeill, Haggarty, Smith, & Franklin, 1997), but higher than that verified in three days of free-living HR monitoring in a limited sample (n=6) by (Grund, Vollbrecht, Frandsen, Krause, Siewers, Rieckert, & Muller, 2000) showing an average within-subject CV of 5.5% (range: 0.8 to 8.5%). However, the reduced sample size and the lack of a gold standard may have compromised the latter finding.

Between-subject CV for TEE predicted by HR-based techniques showed a consistent CV independent of the day combination (that is, all days, weekdays and weekends) for all techniques, except for $\text{FlexHR}_{\text{UBLB}}$ where the CV was increased at weekends. The lowest variability was evidenced by $\text{AH}_{\text{branched}}$, with only a small CV difference for all other techniques used. An average between-participant variability of ~ 20% for all methods was displayed in our sample, results not too different from the CV of 16.7% earlier reported in adults using TEE from heart rate and whole-body calorimetry (Ceesay et al., 1989).

The ICC for all HR-based techniques used to estimate TEE showed reliability of 94, 96, 98, 97 and 96 respectively, for $\text{FlexHR}_{\text{UBLB}}$, $\text{AH}_{\text{branched,}}$ $\text{HRPAnet}_{\text{RMR}}$,

$HRPAnet_{4act}$ and $HRPALBnet_{4act}$, considering all monitored days. Separate analysis of weekdays verified similar reliability for all methods and only slightly reduced differences were found when compared against all monitored days. Our results are similar to the reliability coefficient of 99% from two HR monitoring days reported by Allor & Pivarnik (2001), and reinforce the findings of Durant et al. (1993), indicating that just over 4 days of monitoring was required to estimate children's HR with a reliability of 0.80. This was achieved for all methods used in the present study considering all days or only 5 weekdays. The inclusion of weekend days in the monitoring period had little effect on reliability for all analysed techniques. A number of studies using objective measures of physical activity in youth have documented marked differences in weekday and weekend physical activity behaviour, as described in the previous section. This could be better supported for HR-based techniques, considering the technical characteristics of the gold-standard for energy expenditure measurements widely used today, DLW. A critical mass of DLW data has now been accumulated across a wide range of age groups and body sizes (Brooks et al, 2004). The DLW technique measures the average daily TEE in free-living individuals' across 7 to 21 consecutive days. The disappearance rate of two stable isotopes from body fluids can be used to calculate the carbon dioxide production rate and then calculate TEE. However, we were unable to differentiate between weekdays or weekends in these calculations once TEE was calculated as representative of all measured days. As this method has been used in validation studies of other tools such as heart rate and accelerometers which has shown differences between weekdays and weekends, the inclusion of at least 1 weekend day in the objective measurement of children's physical activity patterns appears advisable.

Analyses for time windows for AEE predicted by HR-based techniques again confirmed that block 1 was the least active time window. In agreement with results from activity counts, block 2 was the most active time window. Significant associations between DLW_{AEE} and block 3 ($r = 0.24$) and block 4 ($r = 0.32$), were verified. Partial correlations controlled by BMI showed associations between DLW_{AEE} and block 3 ($r = 0.26$) and block 4 ($r = 0.33$). Despite no strong correlation these results can be considered as reinforcement of the results previously cited for accelerometer analyses indicating the after-school time window as a period representative of children physical activity. Unfortunately, we are unaware of studies using HR-based techniques to verify AEE in different time windows validated against the DLW technique in children.

Statistically significant associations among all analysed HR-based techniques with DLW_{AEE} were verified. However, when adjusted by weight, only $AH_{branched}$ was associated with DLW_{AEE}. Correlations among DLW_{TEE} and HR-based techniques adjusted by body fat showed significant association with all techniques used but when adjusted by weight associations were only verified for $AH_{branched}$ and $HRPAnet_{RMR.}$ $AH_{branched}$ showed better association with DLW_{TEE} and DLW_{AEE} than other HR techniques, but it is important to note that this technique used a branched model between HR and activity counts. Hilloskorpi et al. (1999) have showed the influence of body weight when predicting AEE using HR regression equations, where increases in body weight also increased AEE during all weight-bearing exercise. When quick changes are made from low to high intensities (and vice versa), the HR response lags behind, introducing error in the TEE prediction (Achten & Jeukendrup, 2003). A possible explanation for the improved associations between $AH_{branched}$ and DLW could be their weighting factors, discriminating between activity and "no-activity" as well as the utilisation of HR thresholds in the presence and absence of activity, helping to verify the increased AEE from weight-bearing activities. Among all techniques, the exclusively HR-based $HRPAnet_{RMR}$ showed the best association with the DLW technique. Iannotti et al. (2004) showed that 73% of the HR values in free-living activities were within the range of the low-level activity. Calibration protocols using treadmill equation only (lower body) may result in an overestimation of daily TEE because a range of low-intensities activities are performed using upper-body throughout a school-day, without locomotion. The design used for $HRPAnet_{RMR}$ may be have minimised this error with the inclusion of the low-level activity performed by the upper body equation. Upper body work will raise the heart rate, but the cardiac output will remain lower than when using larger muscle groups during incremental walking. Hilloskorpi et al. (2003) showed that while smaller muscle groups were being used mean TEE (at mean body weight) at the same HR (bpm) was lower than the TEE of incremental walking. At least in part this calibration model may explain the verified association of $HRPAnet_{RMR}$ with the reference technique. Characteristics of $AH_{branched}$ and $HRPAnet_{RMR}$, as well as the strengths of each design to TEE measurement were previously discussed in sections 4.2.4.2.1, 4.2.4.2.2 and 4.2.4.2.3.

Chapter V – PHYSICAL ACTIVITY PATTERNS IN CHILDREN

5.1 Literature Review

It is important to recognise that physical activity and energy expenditure are not synonymous terms. Physical activity is a behaviour that results in energy expenditure and is typically quantified in terms of frequency (number of bouts) and duration (minutes per bout). Energy expenditure reflects the energy cost or intensity associated with a given physical activity (Lamonte & Ainsworth, 2001). Physical activity is a broadly used term and its heterogeneous nature makes it extremely difficult to characterise and quantify (Molnar & Livingstone, 2000) however it commonly defined as "any bodily movement produced by skeletal muscle that results in energy expenditure" (Caspersen, Powell, & Christenson, 1985).

The assessment of young people's habitual physical activity is very difficult especially because physical changes occur during the years spanning infancy through to young adulthood. Further, many of the available methods are likely to induce behavioural changes in spontaneous and natural activity patterns of those being measured (Molnar & Livingstone, 2000).

The biological basis for the differences in activity patterns between children and adults is associated with children being inherently more active, primarily because physical movement provides them with the necessary information required by the central nervous system for stimulation. In contrast, adults achieve arousal of the central nervous system in a variety of non-locomotor activities such as reading, writing, artistic expression, problem solving and vocational pursuits (Rowland & Green, 1988). The development of techniques that account for differences in size, shape, body composition, level of fitness and maturation, throughout diverse periods of life, is a great challenge (Durnin, 1996).

The search for accurate and reliable field tests of physical activity has become a critical issue for all researchers interested in the prophylactic effects of physical activity. To date, despite voluminous literature in the area and a plethora of field methods available for the assessment of physical activity, no single field measure has proven valid, reliable, and logistically feasible over a wide range of populations, settings, and uses. The prediction of children's physical activity levels is limited by the lack of a 'gold standard' that accurately measures type, quantity, frequency, duration and intensity of daily activity (Ridley, Dollman, & Olds, 2001).

Spadano et al. (2003) observed considerable variability in the measured MET values of activities ranging from sedentary to vigorous intensity among 12-year old girls. These authors reported that body weight explained a large degree of the variation in MET values and suggested that adult METs appear to provide the best estimates of the energy cost of physical activities at this age. Nevertheless, possible bias introduced by differences in body weight should be considered whenever TEE is estimated using average MET values. It is necessary to consider that metabolic constants are provided for a limited number of activities. The literature is scarce in this aspect, suggesting the necessity for more research with children in free-living daily activities. When using the factorial approach there is a need to adjust total energy expenditure from an appropriate metabolic equivalent value as has been shown in adults (Byrne et al., 2005) as well as in children (Roemmich et al., 2000; Harrell, McMurray, Baggett, Pennell, Pearce, & Bangdiwala, 2005).

It is of particular importance to consider the validity and reliability of different methods of physical activity assessment used among children and adolescents. Moreover, an important consideration when evaluating the reliability of measurements of behaviours such as physical activity is an understanding of both the possible error involved with repeated measurements of the same behaviour, and also the error induced by a lack of stability of the behaviour of interest itself, that is, when the behaviour varies over time. In the same way, validation studies of physical activity assessment methods are usually designed to be either indirect or concurrent studies. Concurrent designs use another more precise measure of the same parameter being assessed as validation. On the other hand, indirect validation techniques use a by-product or correlate the physiological property or phenomenon under study as a validation criterion (Kohl et al., 2000).

To accurately assess children's activity patterns an instrument must be sensitive enough to detect, code, or record sporadic and intermittent activity. While we do not currently know which measure is the most accurate, reporting the results with different instruments provides a more complete description of children's activity and permits a triangulation of outcomes (Welk et al., 2000).

It is common to use HR to count the number of minutes spent performing vigorous physical activity and examine time spent in particular HR ranges (Saris, 1986), 1986). The same approach was used by Durant et al. (1993) to investigate the reliability and variability of indicators of heart rate monitoring in children. Since mean daily heart rate is an indicator of overall activity influenced by the fitness level of the child, these authors proposed an alternative index, 'physical activity heart rate – 25 and 50' (PAHR-25, PAHR-50), percentages of heart rates 25% and 50% above resting heart rate. These authors assumed that the PAHR-25 index controls for differences in resting heart rates due to fitness and age differences and reflects most heart rate responses, including light physical activity. Higher within-day and between-day reliabilities have been shown than for mean heart rate. The PAHR-50 index reflects higher activity levels.

Ekelund et al. (2002) reported higher PAL values in a control group were due to both participation in high-intensity activities and a higher daily moderate-intensity activity. The authors suggested that physical activity is not equivalent to the energy cost of activity because the time spent on physical activity may be a more significant factor than energy expenditure attributable to physical activity. The time spent in moderate-intensity physical activity as well as the total amount of physical activity are important concerns. Bailey et al. (1995) contend that children's activities are not only low in intensity but are not sustained over extended periods of time. Molnar and Livingstone (2000) supported this observation and suggested that multiple methods of measurement are valuable together with the DLW technique as this approach does not provide information regarding duration and intensity of physical activity.

Approximately 7 days of measurement is likely to provide a representative assessment of activity thermogenesis. Such 7 day measurement periods can potentially be repeated to better understand the importance of variables such as season or in the case of adults, changing occupational roles (Levine, 2004).

5.2 One size does not fit all

5.2.1 Introduction

Physical activities have been described in the compendium by Ainsworth et al. (2000) as multiples of a standard resting energy value, and MET-based intensity categories have been used to define activity thresholds in adults (Pate & et al., 1995). Physical activity measurement approaches in adults are not always applicable to children, however some researchers have used adult values (Bouchard, Tremblay, Leblanc, Lortie, Savard, & Theriault, 1983). Others have criticised this approach (Torun, 1983) and proposed an age-specific standard fraction to the adult MET (Torun, 1989), however this proposal underestimates the energy cost of simple activities such as walking (Spadano et al., 2003). These authors have suggested that as individual MET values for children are not available, adult MET values appear to provide the best estimates of energy cost, despite the possible bias introduced by differences in body weight. Others have suggested that the compendium of MET values for adults should be adjusted by EE measured in children (Harrell et al., 2005).

One might contend that it is an insurmountable task to determine adjustments to the compendium for children's activities, or to create a specific compendium. The original compendium was determined from multiples sources, and for the vast majority of the activities, values are cited without being based on direct measurements. Some research groups are trying to minimise this problem by using $\dot{V}O_2$ values above measured resting metabolic rate to define activity thresholds (Treuth et al., 2004). Another author has stated that if measured MET values are used in the creation of calibration equations, it may lead to systematic bias in equations for overweight and non-overweight youth (Welk, 2005). A more recent study showed that the mean difference between the measured and predicted MET values were significantly greater for smaller children than for larger children during treadmill walking (Wickel, Eisenmann, & Welk, 2007). Other research groups have avoided this MET category (Puyau et al., 2002; Puyau, Adolph, Vohra, Zakeri, & Butte, 2004).

Cognisant of this brief discussion regarding the adequacy of METs for children, it is important to highlight that the term "moderate-intensity physical activity" refers to activity as multiples of a standard resting energy value. Moderate-intensity refers to energy expenditure three to six times greater than the standard resting metabolic rate in children and is identified as such in physical activity recommendations. However, reference to individually-measured METs is not included in these recommendations (Services, 1996; Cavill, Biddle, & Sallis, 2001; Trost, 2005; Pate, Davis, Robinson, Stone, McKenzie, & Young, 2006). The U.S. Department of Health (http://www.cdc.gov/nccdphp/dnpa/physical/recommendations/young.htm) 'Physical activity for everyone: Are there special recommendations for young people?' provides a link to 'Measuring physical activity' and a table based on CDC and ACSM guidelines: 'General Physical Activities Defined by Level of Intensity'. A footnote to the table verifies that these data are available only for adults. Interestingly, this reference is widely used, for example in the UK Consensus (Cavill et al., 2001), Australian guidelines for children (Trost, 2005) and the American Heart Association statement (Pate et al., 2006) regarding the promotion of physical activity in children and youth. This statement defines "moderate-to-vigorous physical activity" as "…energy expenditure usually at the level of ≥ 3 METs (metabolic equivalent), and the activity expends ≥ 3.5 kcal/min." The inconsistency among children's recommendations is directly aligned with the opinion of Byrne et al. (2005) that when a scientific convention gains widespread acceptance, there is the risk that its underlying premise may no longer be questioned. As a result, the limitations inherent in its assumption are overlooked, and subsequently, the risk of misusing the convention may increase.

The ability to accurately assess moderate-to-vigorous physical activity in free-living conditions is a challenge, particularly as applicable cut-offs are needed in epidemiologic research. However, there is no consensus about the best index to reflect physical activity patterns in children. Accelerometer cut-offs will not be discussed here as they are strongly brand-dependent. There is also concern regarding the transformation of counts to express movement as previously discussed in section 4.2.4.2.7.

Heart rate monitoring has provided a number of options for the measurement of physical activity in children, including the following. Based on a series of studies, Armstrong and associates (Armstrong et al., 1990; Armstrong & Bray, 1991; Biddle, Mitchell, & Armstrong, 1991; Welsman & Armstrong, 1992) defined moderate physical activity as generating a HR ≥ 140 bpm and vigorous physical activity as generating a HR ≥ 160 bpm. A second proposal from Livingstone et al. (1992) defined moderate physical activity as HR > 50% of peak oxygen uptake ($P\dot{V}O_2$) and vigorous physical activity as HR >70% $P\dot{V}O_2$. A third option was proposed by Durant et al. (1993) using percentages of HR 25% and 50% above resting HR and two indices - 'physical activity heart rate' (PAHR-25 and PAHR-50).

In the present study, individual regression equations validated against DLW measurement (section 4.2.4.2) were applied to verify minute-by-minute energy expenditure expressed as multiples of the measured resting energy value. Data across 7 days of monitoring were analysed and quantified by intensity categories and compared with the standard value of 3.5 $ml.kg^{-1}.min^{-1}$ equivalent to 1 MET. The second part of this study analysed each of the three cited heart rate 'cut-offs' together with the measured MET minute-by-minute analyses to verify the agreement between them.

5.2.2 Materials and methods

5.2.2.1 Participants

In the first part of this study, RMR measurement, experiments were undertaken on 26 girls aged 8-12 (mean age = 10.5±1.0 yr, mean mass = 37.6±5.6 kg, mean height = 146±9.0 cm). 19 girls completed all study requirements and the characteristics of participants, eligibility, exclusion criteria and ethical issues were described at section 4.2.2.1.

5.2.2.2 Study Design

Same as 4.2.2.2

5.2.2.3 Data Collection

Same as 4.2.2.3

5.2.2.4 Data Analyses

RMR was assessed using the methodology described in section 2.2.2 from calibration session 1. In the first part of this study, individually-measured resting $\dot{V}O_2$ values were compared with the standard value of 3.5 $ml.kg^{-1}.min^{-1}$ equivalent to 1 MET and 1 $kcal.h^{-1}$. Secondly, data from the treadmill test described in section 4.2.2.3.1.3 were analysed in two different data sets, namely: standard MET ($Stand_{met}$) and individually-measured MET (Ind_{met}), for each speed.

After laboratory-based tests, data from 7 days of monitoring under free-living conditions were analysed minute-by-minute throughout each day. Again, two data sets were constructed, standard and measured METs; with exercise $\dot{V}O_2$ expressed as a multiple of resting $\dot{V}O_2$ during waking hours using the technique $HRPAnet_{RMR}$, detailed in section 4.2. Both data sets were analysed and quantified at three intensity levels: less than 3 METs (light physical activity), between 3 and 6 METs [moderate physical activity (MPA)] and more than 6 METs [vigorous physical activity (VPA)] (Pate et al., 1995).

Briefly, $HRPAnet_{RMR}$ is a technique where regression equations are individually determined to indirectly measure AEE using net $\dot{V}O_2$ ($\dot{V}O_{2exercise}$ minus $\dot{V}O_{2RMR}$) and net HR ($HR_{exercise}$ minus HR_{sleep}) with exercise data from laboratory calibration for lower body and upper body. A regression equation for each exercise type was developed and based on a ratio between accelerometer data mounted at the wrist and ankle ($wrist_{cpm}$ divided by $ankle_{cpm}$) and the relevant equation was chosen for each specific minute of the monitoring time.

An analysis by different time windows with the same methodology used for accelerometers and HR (Section 4.2.2.4) was performed considering time at each intensity category for standard and individual METs.

Physical activity indices

A variety of heart rate indicators have been recommended as valid "tools" to measure children's physical activity patterns. From these we chose three of the most common cut-off points (Table 5.1) to analyse our data.

Table 5.1 – Physical activity indices

Index (MPA)	Index (VPA)	Author
HR ≥ 140 bpm	HR ≥ 160 bpm	Armstrong et al. (1990)
HR > 50% P$\dot{V}O_2$	HR > 70% P$\dot{V}O_2$	Livingstone et al. (1992)
PAHR-25	PAHR-50	Durant et al. (1993)

P$\dot{V}O_2$ = peak oxygen uptake; PAHR-25 & 50 = HR 25% & 50% above resting HR

Time expended in each intensity category was quantified in minutes per day and percentage of the day for every index. Achievement of the daily recommendation of 60 min of MVPA based on each index is detailed in Table 5.1 as well as using Ind_{met} and $Stand_{met}$. Time analyses were performed for all days and separately for weekends.

Bouts

PA bouts at MPA, VPA and MVPA in 6 different time windows (5, 10, 15, 20, 25 and 30 minutes) were analysed for every PA indicator previously cited as well as using Ind_{met} and $Stand_{met}$. Despite the inclusion of one observation below the intensity cut-off point suggested for bout counts using accelerometers (Ward et al, 2005) the criterion in this study was to include only full intervals of consecutive minutes above every cut-off point used in each time window, considering the greater interval as the priority for inclusion in number of bouts. At this time, participants who achieved the recommendation of 60 min of MVPA were analysed by sum of bouts considering all days and weekends.

Statistical analyses

Data is presented as mean values and standard deviations (SD), unless otherwise stated. T-test was used to verify differences between individually-measured and standard values for resting $\dot{V}O_2$ as well as total monitoring time per day and time windows. Relationships between standard and measured MET differences and speed were analysed by linear regression. Repeated measures ANOVA was used to analyse total time in the day, between windows and sum of bout differences by intensities from each index. Values were considered significant at $P < 0.05$. Statistical analyses were carried out with SPSS for Windows (version 15.0, 2006, SPSS, Chicago, IL, USA).

5.2.3 Results

The average resting $\dot{V}O_2$ was 4.7±0.6 ml.kg^{-1}.min^{-1}.; or expressed as energy expenditure, the average value was 0.83 ±0.13 kcal.h^{-1}.

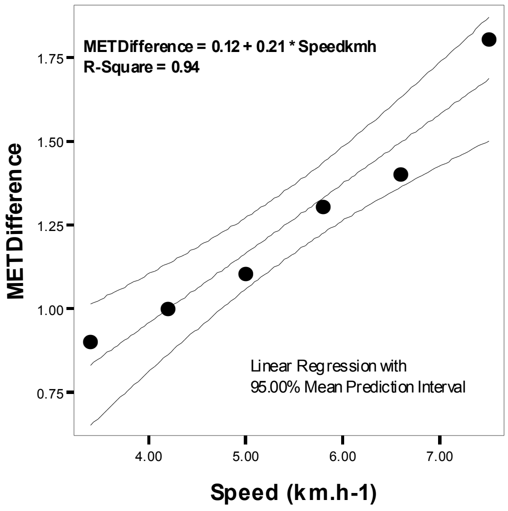
Average (±SD) speed, standard and measured METs on the treadmill are shown in Table 5.2. There was an increasing linear difference between standard and measured METs as a function of the speed (Figure 5.1).

Table 5.2 – METs per speed on the treadmill

Speed	Less 1.6	Less 0.8	SS	More 0.8	More 1.6	More 2.4
km.h^{-1}	3.4±0. 31	4.2±0.31	5.0±0.31	5.8±0.31	6.6±0.31	7.5±0.31
Standard	3.6±0.52*	4.1±0.62*	4.8±0.77*	5.6±0.96*	6.1±1.83*	7.2±2.97*
Measured	2.7±0.53	3.0±0.63	3.6±0.80	4.2±1.00	4.6±1.61	5.3±2.39

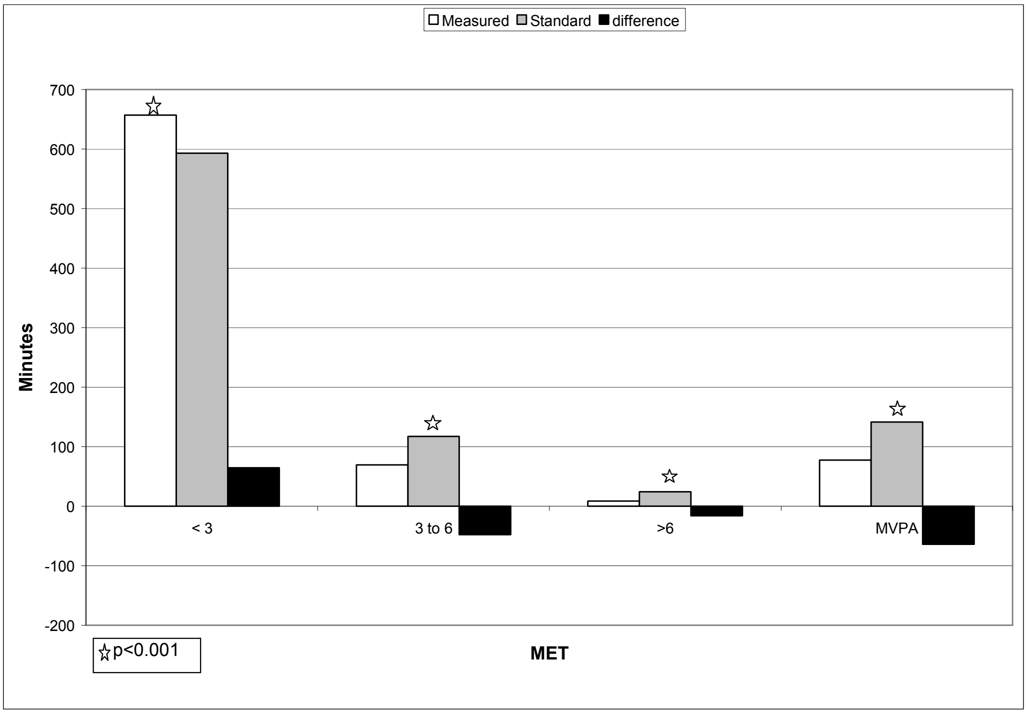
* = Statistically significant different from measured MET ($p<0.001$)

Figure 5.1 – MET differences by speed



Minute-by-minute daily data were quantified by categories including [standard (3.5 ml.kg^{-1}.min^{-1}) and individual (measured) MET] and intensity levels from a mean monitoring time of 735±77 min across 7 days. Average values by categories in each intensity, and difference among them, as well as intensities expressed as moderate to vigorous physical activity (MVPA = 3 to 6 plus >6 METs), are exhibited below (Figure 5.2).

Figure 5.2 – Quantifying METs: Standard versus Measured



An analysis by different time windows with the same methodology used for accelerometers (section 4.2.3.2.1) and HR (section 4.2.3.2.2) were subsequently performed considering time in each intensity category for standard and measured METs (Table 5.3).

Table 5.3 - PA pattern per time window (time and percentage)

	MPA		VPA		MVPA	
Blocks	Measured	Standard	Measured	Standard	Measured	Standard
1	19 (9)	31 (15)	1 (1)	6 (3)	20 (10)	37 (18)
2	28 (13)	41 (19)	3 (2)	8 (4)	31 (15)	49 (23)
3	24 (12)	34 (16)	3 (2)	8 (4)	27 (14)	42 (20)

Paired T-test showed statistically significant differences among blocks between measured and standard METs for all intensities (p<0.001). Repeated-measures ANOVA verified a statistically significant difference between block 2 and block 1 at MPA when using measured METs, with more time at this intensity for block 2

($p<0.05$), but no significant difference from block 3. The same type of analyses using standard METs showed statistically significant differences between block 2 and blocks 1 and 3 at MPA ($p<0.05$), indicating that block 2 was the most active time window of the day. Both analyses showed block 1 as statistically different from block 2 considering minutes expended at intensities below 3 METs ($p<0.05$) as well as no significant difference at VPA among blocks.

Three physical activity indices based on HR were utilised to quantify children's physical activity patterns. It is important to indicate that reference values were used as cut-off points for each index, as well as physiologic data being utilised in this determination (Table 5.4).

Table 5.4 - Mean $P\dot{V}O_2$ and HR data

$P\dot{V}O_2$ ml.kg^{-1}.min^{-1}	HR_{rest} bpm	$HR_{daytime}$ bpm	$HR_{50\%}P\dot{V}O_2$ bpm	$HR_{70\%}P\dot{V}O_2$ bpm	$PAHR_{25}$ bpm	$PAHR_{50}$ bpm
42.1±9.9	74±10	99±9	132±11	160±8	92±12	111±14

Overall mean daytime HR was 26% greater than resting HR ($p<0.001$). Physical activity pattern in minutes and percentages for all 3 indicators are shown in Table 5.5.

Table 5.5 – PA patterns by indicators in minutes and percentages

	≥ 140	≥ 160	$HR_{50\%}P\dot{V}O_2$	$HR_{70\%}P\dot{V}O_2$	$PAHR_{25}$	$PAHR_{50}$
Min	26±20	13±14	40±22	12±11	255±52	198±137
%	3.5±2.7	1.7±1.8	5.3±2.9	1.6±1.5	35.6±7.7	26.7±17.2

Repeated measures ANOVA among these three indicators for MPA and VPA showed no significant difference between HR ≥ 140 X $HR_{50\%}P\dot{V}O_2$ and HR ≥ 160 X $HR_{70\%}P\dot{V}O_2$, but statistically significant differences for $PAHR_{25}$ and $PAHR_{50}$ from the two others indicators at both intensities ($p<0.001$).

Considering this previously identified difference and the extremely high PA time exhibited by $PAHR_{25}$ and $PAHR_{50}$, this indicator was excluded from further analyses. Using Ind_{met} and $Stand_{met}$ from $HRPAnet_{RMR}$, HR ≥ 140 and HR ≥ 160, and $HR_{50\%}P\dot{V}O_2$ and $HR_{70\%}P\dot{V}O_2$ participants who achieved the recommendation of 60 min of MVPA across the 7 days of monitoring was verified. (Table 5.6).

Table 5.6 – PA target of 60 $min.d^{-1}$ by number of day achieved by each index

Days	1	2	3	4	5	6	7
Ind_{met}	3	2	2	1	2	2	1
$Stand_{met}$	1	4	3	3	1	2	5
HR - 140 and 160	5	2	2	0	2	0	0
$HR_{50-70\%}P\dot{V}O_2$	1	2	4	2	1	0	1

The number of children who achieved the target at weekends were as follows: $Stand_{met}$, (n=9), Ind_{met}, (n=5), $HR_{50-70\%}$ $P\dot{V}O_2$ (n=4), and HR- 140 and 160 (n=1).

Earlier in this chapter we showed minute-by-minute analyses using individual and standard METs from the $HRPAnet_{RMR}$ technique to quantify time for activity intensity. We subsequently added time spent at each activity intensity (MPA, VPA and MVPA) to the analysis from HR – 140 & 160 and $HR_{50-70\%}P\dot{V}_{O2}$.

Considering intensities between 3 and 6 METs there was no statistically significant difference between Ind_{met} and $HR_{50\%}P\dot{V}O_2$. For intensities greater than 6 METs, no statistically significant difference was verified between Ind_{met} and $HR_{70\%}P\dot{V}O_2$ or Ind_{met} and HR≥ 160. The same types of analyses performed using the $Stand_{met}$ showed statistically significant differences among all indicators.

Physical activity bouts at MPA, VPA and MVPA in 6 different time windows (5, 10, 15, 20, 25 and 30 minutes) were analysed for every PA index as well as for Ind_{met} and $Stand_{met}$. Children who achieved MPA and VPA in each bout-time by index (percentage in brackets) are shown in Tables 5.7 and 5.8, respectively.

Table 5.7 – Children in each bout-time at MPA by index

Bouts	5	10	15	20	25	30
Ind_{met}	18 (95)	16 (84)	10 (53)	9 (47)	8 (42)	6 (32)
$Stand_{met}$	19 (100)	18 (95)	16 (84)	12 (63)	7 (37)	13 (68)
HR ≥ 140	19 (100)	11 (58)	6 (32)	3 (16)	0 (0)	3 (16)
$HR_{50\%}P\dot{V}O_2$	19 (100)	15 (79)	10 (53)	4 (21)	5 (26.3)	3 (16)

Table 5.8 – Children in each bout-time at VPA by index

Bouts	5	10	15	20	25	30
Ind_{met}	8 (42)	3 (16)	1 (5)	0 (0)	1 (5)	0 (0)
$Stand_{met}$	12 (63)	7 (37)	3 (16)	1 (5)	0 (0)	2 (10)
HR ≥ 160	10 (53)	5 (26)	2 (10)	1 (5)	1 (5)	0 (0)
$HR_{70\%}P\dot{V}O_2$	11 (58)	5 (26)	1 (5)	1 (5)	1 (5)	1 (5)

Bouts were summed and participants who achieved the recommendation of 60 min of MVPA for 7 days of monitoring were quantified (Table 5.9). The same analysis was performed for weekends (Table 5.10).

Table 5.9 – PA target of 60 $min.d^{-1}$ achieved from sum of bouts and number of days

Days	1	2	3	4	5	6	7
Ind_{met}	3	1	3	1	0	2	0
$Stand_{met}$	6	3	1	1	2	1	2
HR - 140 and 160	5	1	0	0	0	0	0
$HR_{50-70\%}\ P\dot{V}O_2$	6	2	0	1	0	0	0

Table 5.10 - PA target of 60 $min.d^{-1}$ achieved from sum of bouts at weekends

Intensities	MPA	VPA	MVPA
Ind_{met}	3 (16)	0 (0)	3 (16)
$Stand_{met}$	4 (21)	3 (16)	7 (37)
HR- 140 & 160	0 (0)	0 (0)	0 (0)
$HR_{50-70\%}\ P\dot{V}O_2$	0 (0)	0 (0)	0 (0)

Comparisons among exercise time (sum of bouts) at MPA showed significant differences ($p<0.05$) between Ind_{met}, $\text{Stand}_{\text{met}}$, HR $\geq$ 140 and $\text{HR}_{50\%}\ \text{P}\dot{\text{V}}\text{O}_2$. At VPA, a significant difference ($p<0.05$) was only found between Ind_{met} and $\text{Stand}_{\text{met}}$.

5.2.4 Discussion

5.2.4.1 Measured *versus* standard METs

The origin of the value of 3.5 $ml.kg^{-1}.min^{-1}$ equivalent to 1 MET is vague. This value is commonly accepted for adults and is reported in most scientific textbooks; however recently there has been some critical debate regarding the use of this standard figure for all people, irrespective of size. Byrne et al. (2005) have shown that this standard value overestimates the actual resting $\dot{V}O_2$ value on average by 35%, and the 1-MET of 1 $kcal.h^{-1}$ overestimates resting energy expenditure by 20%.

Standard values for resting $\dot{V}O_2$ in this study showed an underestimate of 33% and the 1-MET of 1 $kcal.h^{-1}$ overestimated resting energy expenditure by 17% in children. The resting $\dot{V}O_2$ of younger children has consistently been reported as higher than adults (Boothby, Berkson, & Dunn, 1936; Schofield, 1985), with a steady decrease from the age of 6 years to adulthood.

In all 4 walking and 2 running speeds on the treadmill the use of the standard MET showed an overestimate compared to the measured MET, with an increase in difference linearly related to speed increments. Harrell et al. (2005) have suggested that a way to adapt the adult compendium of physical activities (Ainsworth et al., 2000) for use in children is to adjust the estimated EE for the higher resting EE. We are able to compare 2 of our mean walking speeds with similar speeds used by these authors (4.0 and 5.6 $km.h^{-1}$ versus 4.2 and 5.8 $km.h^{-1}$ in the present study). Despite the small differences in speed, the work of Harrell et al. (2005) showed higher mean MET values than in the present study. The walking speeds of 4.0 and 5.6 $km.h^{-1}$ equate to 3.2 and 4.3 METs compared with 4.1 and 5.7 METs in the present study. We believe that resting EE would explain differences verified during treadmill walking between studies. The study by Harrell and colleagues showed for girls of the same age, the mean resting $\dot{V}O_2$ of 5.9 $ml.kg^{-1}.min^{-1}$ with adjusted resting EE to the standard value of 1.71±0.41 ($kcal.kg^{-1}.h^{-1}$). In the present study the mean resting $\dot{V}O_2$ was 4.7 $ml.kg^{-1}.min^{-1}$ with an adjusted resting EE of 1.33±0.17 ($kcal.kg^{-1}.h^{-1}$). Potentially, methodological differences in REE measurements discussed in Chapter 2 may help to explain the different results between samples. Harrell et al. (2005) measured REE after 5 min rest for 15 min. The first 5 min of the measurement as well as the last minute were eliminated, and therefore REE was obtained from the average of 9 min. Perhaps 5 min rest is not sufficient for children to achieve the required steady state to reduce error and ensure accuracy of measurements (Reeves et al., 2004). Steady state is often defined as 5 consecutive minutes during which oxygen consumption and carbon dioxide production vary by ±10%, as used in our measurements (see section 5.2.2.4). However, independent of the methodological differences both studies are in agreement that the use of the standard MET is not adequate for children. Across a range of speeds our study showed increased differences directly proportional to the exercise intensity.

Among all techniques from section 4.2.2.4.1 we are able to verify EE on a minute-by minute basis throughout the day from an individualised regression equation. $\text{HRPAnet}_{\text{RMR}}$ showed the most significant association with DLW_{TEE} and DLW_{AEE} ($p<0.01$) as well as when adjusted for body weight ($p<0.05$). Importantly, $\text{HRPAnet}_{\text{RMR}}$ when compared with DLW measurements, showed a mean difference of 67.2 kcal.d^{-1} as well as a standard error of the estimate of 99.2 kcal.d^{-1}, equivalent to an error of only 4.5% against the gold standard technique.

Minute-by-minute analyses of 7 days of free-living monitoring using $\text{HRPAnet}_{\text{RMR}}$ showed average values for measured-METs as 657 min below MPA (< 3 METs), 69 min of MPA (3 to 6 METs) and 8 min of VPA (> 6 METs). However, the standard MET showed a twofold overestimation of MPA (117 min) as well as a threefold overestimation for VPA (24 min). As expected, the largest part of the day was classified at intensities < 3 METs for both categories. However, standard values underestimate this lowest intensity by 64 minutes, consequently spreading this difference between the next two intensity categories – MPA and VPA for a total overestimation of 64 minutes considering activities higher than 3 Mets (MVPA). For all intensities these difference were statistically significant ($p<0.001$). It is interesting to note that the verified overestimation using widespread standard value is exactly the daily recommendation for physical activity in children (Sallis & Patrick, 1994; Services, 1996; Biddle, Cavill, & Sallis, 1997; Trost, 2005).

We are not aware of any study that has used the HR technique validated against a reference method conducted in free-living conditions to compare the measured and standard MET in children. A study using a specific accelerometer-based MET equation against indirect calorimetry showed a difference between the measured and predicted MET values across a range of treadmill walking speeds. This was directly dependent on the body surface area and the predicted MET was significantly greater for smaller compared to larger children (Wickel et al., 2007). Spadano et al. (2003) have reported that the extent of over- or underestimation of TEE due to body size will depend on the relative contribution of each activity performed in an individual's TEE and the degree of influence body weight has on MET values of each of the activities. Another study conducted under laboratory conditions analysed a specific set of activities commonly performed by children and underlined the inadequacy of standards determined for adults used in measurements with children (Harrell et al. 2005). Despite between-study design differences, a reasonable conclusion is that individual measurements of RMR are needed to establish individual MET values if one wants to express physical activities as multiples of resting energy values and make further comparisons with physical activity intensity MET-based categories to define activity thresholds. The use of this procedure could help to avoid overestimates of children's physical activity level.

Time window analyses showed statistically significant differences among blocks between measured and standard METs for all intensities ($p<0.001$). Standard METs overestimated time in MVPA from block 1 to block 3 as 16 min (7.6%), 17 min (8.3%) and 14 min (6.7%), respectively. The sum of the overestimation for each block was 47.5 min, representing 79% of the 60 min.d^{-1} recommendation for children. If one only uses the standard MET to analyse different time windows, one could draw erroneous conclusions about the physical activity pattern of children. Despite the emerging proposal for time window evaluation (O'Connor et al., 2003; Hesketh et al., 2007) as estimates of PA pattern in children are interesting our results underline the importance of utilising the measured MET and that the risk of under- or overestimation is directly dependent on which time window is measured.

5.2.4.2 Physical activity patterns

Analyses of children's physical activity patterns by intensity levels across 7 days in free-living conditions was performed from each proposed index. There were no statistically significant differences between HR ≥ 140 and $HR_{50\%}P\dot{V}_{O2}$ (MPA) as well as between HR≥ 160 and $HR_{70\%}P\dot{V}_{O2}$ (VPA). However, statistically significant differences for $PAHR_{25}$ and $PAHR_{50}$ from two others indices at both intensities were verified. The impact of different definitions of resting HR on the activity level of children was analysed by Logan et al. (2000). Depending on the protocol, $PAHR_{25}$ varied by 10-50% and $PAHR_{50}$ varied by 16-65%. Potentially, our protocol regarding sleep heart rate impacted negatively on the findings from $PAHR_{25}$ and $PAHR_{50}$. From this initial analysis of results, and also with the very high values at each intensity level, this latter index was eliminated from other analyses. Table 5.11 summarises results from a range of studies in girls of a similar age as well as number of participants, number of days monitored, criterion for MVPA, results expressed minutes/day and country where the study was conducted.

Table 5.11 – Studies using heart rate as a criterion for MVPA in girls

Study	n	Days	Criterion	Result	Country
Armstrong et al. (1990)	11	3	≥ HR140	45.4	UK
Armstrong et al. (1991)	13	3	≥ HR140	34.9	UK
Welsman & Armstrong (1992)	45	3	≥ HR140	30.0	UK
Armstrong & Bray (1991)	65	3	≥ HR140	67.7	UK
Sallis et al. , (1993)	18	1	≥ HR140	42.7	US
Gilbey & Gilbey (1995)	64	4	≥ HR140	30.5	Singapore
Falgairette et al. (1996)	34	7	≥ HR140	51.1	Belgium
Sallo & Silla (1997)	29	1	≥ HR140	50.0	Estonia
Rowlands et al. (1999)	10	1	≥ HR140	34.2	UK
Calvert et al. (2001)	10	3	≥ HR140	41.8	NZ
Al-Nakeeb et al.(2007)	24	3	≥ HR140	54.7	UK
Present study	**19**	**7**	**≥ HR140**	**39.0**	**Australia**
Livingstone et al. (1992)	9	2-3	$50\%P\dot{V}O_2$	39.0	Ireland
Durant et al. (1993)	66	1	50%HRR*	68.8	US
Gavarry et al. (1998)	19	7	50%HRR*	91.3	France
Ekelund et al. (2000)	40	3	$50\%P\dot{V}O_2$	42.0	Sweden
Present study	**19**	**7**	$\mathbf{50\%P\dot{V}O_2}$	**52.0**	**Australia**

* %HRR ~ $\%\dot{V}_{O2max}$ (ACSM, 2006)

Considering the time spent with HR ≥ 140 as an MVPA indicator, the average time was 39±34 min representing 5.2%±4.5% of the total monitoring time averaged from

7 days of monitoring. Our results are similar to those of Armstrong and collaborators in a series of studies in a large cohort over 3 schooldays (Armstrong, 1998). In the same age interval as in the present study, it was shown that girls spent 7.7% of the time with their HR $\geq$ 140 bpm. A sub-sample measured during 1 weekend day showed 6.0% of the time with HR $\geq$ 140 bpm. Recently, an average time of 7.6% using the same criterion was verified in girls of a similar age by Al-Nakeeb et al. (2007). However, each of these studies only considered three days of monitoring which may not be representative of the 'real' physical activity pattern of these children. Trost (2001) stressed that 7 days of monitoring produced acceptable estimates of daily MVPA and accounted for significant differences in weekday and weekend physical activity.

Analyses of the MVPA using the time spent and the HR > $_{50\%}P\dot{V}_{O2}$ indicator showed an average time of 52±32 min representative of 6.9%±4.4% of the total monitoring time. This result is in close agreement with the 49±20 min reported earlier by Livingstone et al. (1992). The percentage of $\dot{V}O_{2max}$ is generally equivalent to the percentage of heart rate reserve (HRR) (ACSM, 2006) allowing comparisons with a meta-analysis using a quantitative review of heart rate measured activity in youth (Epstein, Paluch, Kalakanis, Goldfield, Cerny, & Roemmich, 2001). Considering only the age interval of the present study, the minutes of physical activity, considered here as the sum of 50% to 60% HRR and 60% to 70% HRR, showed 48.6 minutes at MVPA, surprisingly the same time as found by Livingstone and very close to that in the present study. Comparing average time spent at MVPA between HR $\geq$ 140 bpm and HR > $_{50\%}P\dot{V}_{O2}$ a coefficient of variability of 20% was verified.

It is extremely interesting to consider the findings presented in table 5.11. First, considering the average value from all cited studies, one could contend that children around the world are spending approximately 48.3 min.d^{-1} at an adequate intensity of physical activity (MVPA). Therefore, a logical extension of this finding is that children are 20% below the recommended target of at least 60 min.d^{-1}. For some individuals this may be one of the reasons for the escalating obesity rate and other health problems in children.

However the ability to generalise is limited because some of these studies may not have used representative samples but analyses of variability between-indices can provide useful information regarding the tools used to assess the physical activity pattern of children. From the average of each index cited in Table 5.11, a coefficient of variability of 21% was established, values similar to the present study. These results indicate that HR $\geq$ 140 bpm consistently estimated lower MVPA time than HR > $_{50\%}$P$\dot{V}_{O2}$. In the present study, statistically significant differences were verified among all indices measuring MPA, but no differences were verified in VPA measurements, except between Ind$_{met}$ and Stand$_{met}$. However, whether each of these PA indices is under- or overestimating the time at MVPA is still debatable due to the lack of a gold standard.

The recommended PA target of 60 min.d^{-1} is meant to be achieved on most if not all days of the week. Each index classified a different number of participants as achieving this daily target. By considering any 5 days from the 7 days measured the target was achieved by 5, 1, 1 and no children, respectively for Stand$_{met}$, Ind$_{met}$, HR$_{>50}$P$\dot{V}_{O2}$ and HR >140bpm.

Choosing the most adequate index to verify physical activity patterns is still problematic as no suitable 'gold standard' criterion measure exists for all physical activity comparisons. Despite the outcome of different 'tools' being expressed as MVPA, some of them were based on differing dimensions of physical activity. For this reason, comparisons of MVPA data between different studies should recognise the distinctions between aspects of physical activity assessed (Fairclough & Stratton, 2005). Both indices used in the present study are based on a physiological response to exercise, a measure of the relative stress being placed on the cardiopulmonary system by the activity (Armstrong et al., 1990). It is interesting to note that the mean value for HR equivalent to 70% of the P$\dot{V}O_2$ in the present study was exactly the same value as the cut-off proposed by Armstrong et al. (1990) for more than 6 METs. Using the same criterion of HR equivalent to 70% of the P$\dot{V}O_2$, Livingstone et al. (1992) found a mean HR of 165 bpm for girls of a similar age. The loss of the linearity between HR and $\dot{V}O_2$ in more intense activities can be attributed to the achievement of the ventilatory or lactate threshold but generally the anaerobic threshold of children occurs at a higher percentage of $\dot{V}O_2$ max than in adults (Nixon, 2000). This can, at least partially, explain the ability of both indices to quantify VPA in children. However at intensities between 3 and 6 METs the utilisation of a more individualised index such as $HR_{50\%}P\dot{V}_{O2}$ was a better alternative index than a criterion based on a general HR cut-off. This can be influenced by individual fitness level considering that unfit compared to better trained individuals have a higher heart rate at a given $\dot{V}O_2$ (Montoye et al., 1996).

It has been suggested that the total time, or percentage time heart rate is above certain pre-selected thresholds could be more informative if the number and length of the sustained period (in bouts or bursts) could be noted (Armstrong et al., 1990; Janz, Golden, Hansen, & Mahoney, 1992; Armstrong, 1998)). Analyses of bouts showed that sustained periods of PA are not characteristic of children's PA patterns as reported in the literature (Armstrong et al., 1990; Gilbey & Gilbey, 1995; McManus, Armstrong, & Williams, 1997; Sallo & Silla, 1997; Armstrong, 1998). The most common bout period of MPA was 5 min, achieved by almost 100% of the sample irrespective of the indicator used. However for all bouts greater than 5 min, a decrease in participation was found with increased variability among indicators after 10 min bouts, which were only sporadically achieved. Bouts of 5 min for VPA were achieved by around 50% of the sample, with an increased reduction at 10 min bouts and sparse participation in all bout periods after this time. To illustrate bout distribution at MPA and VPA, HR 140 (Figure 5.3) and HR 160 (Figure 5.4) were used as examples.

Mean MVPA daily-time from minute-by minute analyses spent with HR ≥ 140 was 39±34 min and for HR > $_{50\%}P\dot{V}_{O2}$ was 52±32 min across all monitored days. Mean individual MVPA, considering the sum of bouts was 17.3±19 min for HR ≥ 140 and 19.7±16.6 min for HR > $_{50\%}P\dot{V}_{O2}$. These results evidenced that two-fold less time was spent at MVPA when considering daily-time from the sum of bouts, independent of the index used.

Using the sum of bouts one can verify the number of participants who achieved the PA target of 60 $min.d^{-1}$. When the sum of bouts was compared with the target of 60 min.d^{-1} from total time minute-by minute analyses a reduction in the number of participants classified as adequately active was found.

Figure 5.3 – Bout distribution at MPA

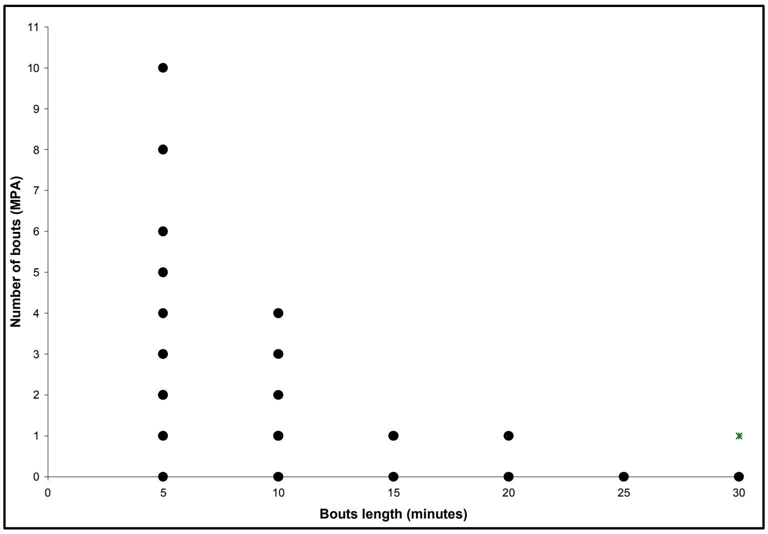
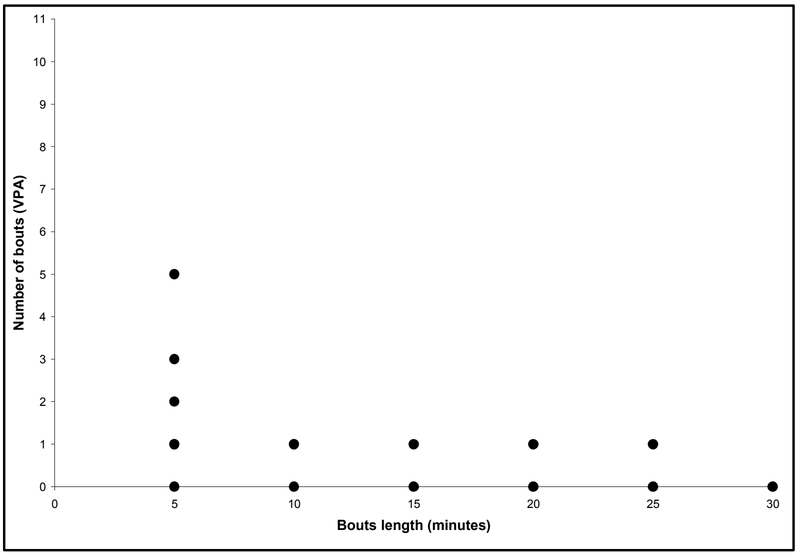


Figure 5.4 – Bout distribution at VPA



The benefits of accumulated intermittent exercise has been well documented for adults (Services, 1996). The concept of accumulating intermittent activity is clearly consistent with children's normal movement patterns (Welk et al., 2000). The intermittent nature of children's activity has been very well characterised, including the observational study by Bailey et al. (1995) and a more recent study by Baquet et al. (2007), Children's activity patterns are sporadic and characterised by shorter and more explosive bouts. However, additional advantages of physical activity at MVPA expressed as a proportion of total accumulated time throughout day and the beneficial consequences of acute, short and repeated bouts on children's health are still to be explored.

The interpretation of the physical activity level of children depends in large part on the criterion levels of activity adopted (Livingstone, Robson, Wallace, & McKinley, 2003). In agreement with this premise we showed that PA patterns are not consistently comparable with different indices and that a variety of results are possible from the same data set. To evaluate MVPA in children under free-living conditions it is recommended that a more individualized index be utilised, for example, $HR_{50\text{-}70\%}P\dot{V}O_2$. A PA threshold based on simple and general HR cut-offs appear to be more suitable for VPA assessment.

Physical activity pattern is a behaviour which is extremely difficult to characterise given the wide within- and between-day variability plus the infinite range of movement possibilities throughout each day. To illustrate this situation we have to compare PAL data from section 4.2.3.2 where 73.6% of our sample was classified as adequately active. However, using a range of indices very few children achieved the physical activity recommendation of 60 $min.d^{-1}$ on most days of the week. These contradictory findings for the same sample are possible because there is no gold-standard technique with the ability to measure all dimensions of physical activity in free-living conditions and thereby specifically quantify physical activity patterns.

Chapter VI – CONCLUSIONS

6.1 General discussion

The primary objective of this thesis was to identify a suitable indirect and objective measurement technique for energy expenditure and physical activity patterns in children based on reproducible measures and validated against a criterion method. To achieve this goal a series of methodological studies were conducted in the first part of the program of work (Chapters II and III). This work was necessary to increase accuracy during the individualised laboratory calibration process and further minimise prediction errors when analysing data from 7 days of monitoring under free-living conditions in the second part of the study (Chapters IV and V). Major examples of originality of this body of work include the use of a multi-system approach to measure movement using 4 accelerometers mounted on different body locations and synchronised with heart rate data. This was undertaken across a 7 day period under free-living conditions and validated against a reference method. In addition, for the first time, minute-by-minute data from individually-measured METs in children were analysed to quantify moderate-to-vigorous physical activity and this was compared with a number of the more common physical activity indices to verify whether children achieved the recommended physical activity target of 60 minutes per day.

Chapter II of this thesis dealt with the measurement of RMR. Knowledge of an individual's energy needs is essential to develop an appropriate diet and physical activity prescription as well as to minimise errors in energy expenditure measurements or predictions. It is important to consider that RMR in general is within 10% of the basal metabolic rate, but for practical reasons, RMR is widely used. It is recommended that RMR be measured in children whenever possible because pediatric RMR equations only explain 52 to 89% of the RMR variability (Henry et al., 1999). RMR is the major component of total energy expenditure in children and is widely considered to be responsible for approximately 60-70% of total daily energy expenditure, especially when a sedentary lifestyle is predominant.

In the current study, RMR accounted for 56.4±11.7% of TEE, with a between-participant variability of 21%.

RMR has been applied as a tool in a range of measurement approaches including PAL, an epidemiologic index described by the ratio of TEE to RMR (TEE/RMR) (WHO, 1985); in exercise prescriptions considering oxygen uptake reserve defined as $\dot{V}O_2$ at a given workload minus resting $\dot{V}O_2$ (ACSM, 2006); in nutrition studies to compare the energy cost of various physical activities described as a physical activity ratio (James & Schofield, 1990); as net $\dot{V}O_2$ to quantify energy cost of physical activity above rest (Andrews, 1971), and to describe physical activity patterns considering individually-measured METs as shown in the present study. Schutz et al. (2001) advised that it is important to be aware that the use of a ratio to adjust biological parameters poses some risk that can result in mathematical errors. We can add that the measurement precision in RMR is directly dependent on a combination of methodological factors which may increase errors when RMR is applied as a ratio.

In particular, results of the first study (section 2.2) showed that there were no differences in RMR measurements due to body position (seated or lying) and apparatus (facemask and mouthpiece/nose-clip) when each variable was isolated. Analyses of distraction (TV or no TV) in three of four combinations among body position and apparatus indicated no difference between TV viewing or rest alone. However, significant differences were verified when analysing distraction combined with seated body position and using a facemask. It is necessary to be cautious when comparing RMR measurements in children in studies that have used different combinations of approaches. Different combinations can result in increased bias and variability in reported differences among children’s RMR measurement. When measuring RMR in children we hypothesised that maintenance of a resting state is likely to be enhanced by using TV watching to reduce boredom. Our study participants indicated a preference for the face mask as well as watching TV during RMR measures despite there being no significant effect of either protocol (apparatus or distraction) on values. However, to reduce the burden in research or in clinical measurements involving children, the combined objective and subjective data from this study provides evidence that the utilisation of a facemask and watching TV should be considered as an effective approach to be used when designing RMR measurement protocols.

Two fundamental principles must be considered in measurement and evaluation, validity and reproducibility. Methodological investigations were undertaken to verify within- and between-day variability and reliability of key variables used in the free-living monitoring part of this study. Specifically, issues included what would affect the prediction of total energy expenditure such as submaximal $\dot{V}O_2$, heart rate, gross economy and substrate oxidation at a range of speeds based on the SS. When employing speeds greater than and less than this reproducible speed reference, a valid and reliable analysis of the energy cost and substrate utilisation was possible. Walking is a common activity of daily life and the range, 3-6 $km.h^{-1}$, as used in this study, has been used as a reference for walking speeds habitually used for transportation (Brooks et al., 2004).

Activity thermogenesis can be separated into two components, exercise-related activity thermogenesis and "non-exercise activity thermogenesis" (NEAT). NEAT is the most variable component of energy expenditure, contributing substantially to inter- and intrapersonal variability and is dramatically affected by environmental factors, mainly those promoting a sedentary lifestyle (Levine, 2004). It is recognised that even trivial movements may be associated with substantial deviations in energy expenditure above resting values. Very low levels of physical activity can increase energy expenditure above resting levels by 20-40% (Levine et al., 2000). It is not surprising, therefore, that during ambulation when body weight is supported and translocated, there are substantial excursions in energy expenditure (Haymes & Byrnes, 1993). Purposeful walking (3 – 5 $km.h^{-1}$) is associated with doubling or tripling of energy expenditure implying that ambulation may be a key component of NEAT (Levine, 2004).

Motivated by the literature to date, the third chapter of this thesis consisted of a sequence of studies about determinants of walking energy expenditure in children. The first paper (section 3.2) dealt with treadmill adaptation and the determination of

SS. Assessment of individual and group differences in metabolic energy expenditure using oxygen uptake requires that individuals are comfortable with, and can accommodate to, the equipment being utilised. To ensure that data from a treadmill walking protocol does not simply reflect an adaptation to the test, it has traditionally been assumed that participants should be habituated to walking on the treadmill before testing (Maltais et al., 2003). Participants with insufficient exposure to the apparatus may be unable to make the necessary adjustments to achieve a stable and consistent gait pattern and therefore introduce bias to the measurements (Frost et al., 1995). The first part of this section was comprised of a detailed description of an adaptation protocol based on the SS. Our results from three treadmill tests following the utilisation of this adaptation protocol showed similar results for step length with no significant differences among tests and lower and no statistically significant variability within- and between-day.

The SS should be determined using a consistent protocol to ensure that the representative walking speed of an individual is identifiable and subsequently applied in walking projects. Our results showed very good correlations and no statistically significant differences between SS determined over-ground and on a treadmill. These results suggest that SS speed determined over-ground is reproducible on a treadmill and our 10 min familiarisation protocol based on this speed was able to provide sufficient exposure to achieve accommodation to the treadmill.

The second paper (section 3.3) of chapter III aimed to investigate the within- and between-day repeatability and variability in children's physiological responses during treadmill walking using the preferred speed and three derived velocities. Repeatability within and between-day for oxygen uptake, gross economy and heart rate for all speeds was verified, suggesting that submaximal exercise can be stable and reproducible at a range of speeds based on children's SS. The lowest variability for oxygen uptake was evidenced when children walked at a comfortable speed (SS) or at a close but lower velocity (0.8 $km.h^{-1}$ less than SS). Results showed a U-shaped curve among CV by speed in the same way that can be seen when gross economy is plotted versus walking speed. The utilisation of SS was undertaken to reflect the speed an individual would select during the common walking tasks of everyday life (Browning & Kram, 2005).

The objective of the third paper (section 3.4) was to verify the relationship between substrate utilisation and exercise intensity expressed as velocity based on the self-selected walking speed in girls and to assess whether this relationship could be reproduced. The fat oxidation difference was verified, expressed as both absolute and relative values, between the 2 slow (V1 and V2) and 2 fast speeds (V3 and V4) with the greatest fat oxidation in the two fast speeds but with no significant difference between them. These findings indicate that the self-selected speed (V3) promotes higher fat oxidation rates than more intense exercise which adds no further advantage in terms of fuel utilisation. It is relevant to acknowledge that the reproducibility expressed as ICC was acceptable and statistically significant for $\dot{V}O_{2,}$ $\dot{V}CO_{2,}$ and absolute and relative CHO and fat oxidation. Analyses of trials at each velocity showed no significant between-day substrate use differences.

The reliability of the submaximal oxygen uptake and substrate oxidation in children walking at a range of comfortable speeds are key findings for indirect energy expenditure measurement in free-living conditions using indirect techniques, as performed in Chapter IV of this thesis. Walking is an important determinant of functional capacity and related daily physical activities whereby most activities of daily living represent exertion at a submaximal exercise capacity.

Commonly, exercise intensity is expressed as a percentage of maximal (or peak) oxygen uptake and comparisons of substrate oxidation at fixed percentages of $\dot{V}O_{2max}$ are undertaken to 'normalise' for level of cardiorespiratory fitness. However, it has been shown that the same relative intensity corresponds to a wide range of exercise intensity. The equivalence of a specific relative proportion of maximal aerobic power in terms of substrate oxidation may be questioned. Self-selected walking speed is commonly identified as the most efficient walking speed; with increased efficiency defined by lower $\dot{V}O_2$ per unit mechanical work (Hoyt & Taylor, 1981; Taylor et al., 1982; Hreljac, 1993). In the final section of Chapter III (section 3.5) it was hypothesised that a valid and reliable analysis of differences in the energy cost and substrate utilisation of different cohorts when exercising, in this case girls and adult women, would be possible when employing speeds greater than and less than this reproducible reference.

This study showed that in both groups, SS was the most economical speed considering the amount of energy consumed per unit of distance. However girls were less economical than women at all of the speeds assessed. From the perspective of substrate utilisation, the shape of the fat oxidation curve for girls was different from that of the adult women. Fat utilisation in adults achieved a plateau at a relative velocity 0.8 $km.h^{-1}$ slower than the self-selected speed, but for girls, fat utilisation increased until the self-selected speed then stabilised until reaching the higher velocity studied. CHO oxidation curves rose abruptly above V2 for women, while for girls the acute part of this increase occurred after the self-selected speed (V3). Collectively, these results indicate that as walking intensity increases, girls are able to meet the energy demands of the work by increasing fat oxidation together with the increased CHO oxidation up to the self-selected speed. In contrast, for adult women, increasing CHO oxidation is associated with an early decrease in fat utilisation at a velocity slower than the self-selected speed.

The second part of this thesis, from Chapter IV (section 4.2) dealt with the validation of indirect techniques for the measurement of energy expenditure in free-living conditions against the reference technique. Objective techniques used included accelerometry, heart rate monitoring and the simultaneous use of both, and a variety of calibration equations from low to high intensity activities, and considering the involvement of both upper-body and lower-body. Further, we controlled for the elevation in heart rate due to non-exercise reasons using a branched equation. Filters were inserted for data cleaning and the approach was validated against the doubly labelled water technique as an alternative to energy expenditure prediction in free-living monitoring in children.

To indirectly predict energy expenditure, 12 different procedures (section 4.2.2.4.1) were used. Among these, only $AH_{branched}$ was based on a group equation, each of the other techniques were based on individualised regression. However, no significant differences between predicted ($AH_{branched}$) and measured TEE using DLW were verified (SEE of 79 kcal.d^{-1}) which represented 3.6% of the mean group measured TEE). On the other hand, the mean difference was 72 kcal.d^{-1}, a mean individual difference of -4.4% with the 95% CI ranging from -238 to 93.9 kcal.d^{-1}, or -10.8 to 4.3%. These results suggest that $AH_{branched}$ combining an activity and HR algorithm, provided similar estimates of TEE in children under free-living conditions from both a group and an individual perspective.

Three of the individually-based techniques used were able to accurately predict energy expenditure in free-living conditions, namely $HRPAnet_{RMR}$, $HRPAnet_{4act}$ and $HRPALBnet_{4act}$. $HRPAnet_{RMR}$ was only slightly more accurate than $HRPAnet_{4act}$ and $HRPALBnet_{4act}$, however this technique is only adjusted by RMR while the other two are heavily dependent on more complex laboratory calibration. No significant differences between $HRPAnet_{RMR}$ predicted and measured TEE using DLW were verified, showing a SEE of 99 kcal.d^{-1} or 4.5% of the mean group measured TEE. The mean difference verified was -67 kcal.d^{-1} representing a mean individual difference of -3.0% with a 95% CI ranging from -276.6 to 141.9 kcal.d^{-1}, representative of -12.6 to 6.5%.

Both techniques, $AH_{branched}$ and $HRPAnet_{RMR}$ were verified as valid and similarly suitable for the prediction of energy expenditure in children's free-living conditions. A preference for one over the other would depend on available resources. Both techniques are dependent on laboratory calibration however $AH_{branched}$ has the advantage of the utilisation of a single treadmill test for HR and activity calibration as well as the device being small and discrete with no interference to daily activities of participants. In contrast, $HRPAnet_{RMR}$ is dependent on two different calibration routines to determine specific regression equations for the upper and lower body as well as the use of 4 devices, an ankle-mounted accelerometer, another at the wrist plus a HR transmitter and receiver.

However, the use of a multi-system approach for $HRPAnet_{RMR}$ is interesting if one needs more detailed information about physical activity patterns in children, or to quantify the use of specific body movements, which can be important when trying to verify sedentary behaviour. The main drawback of $AH_{branched}$ is the cost of the Actiheart device. However when compared to the need for multiple devices to calculate $HRPAnet_{RMR}$, this limitation may be nullified. It is important to consider that there is a range of accelerometers and HR monitors available and that these tools are widely used in laboratory studies of energy expenditure. In many cases, the acquisition of a new device may not be necessary and enable the use of $HRPAnet_{RMR}$ without additional cost.

A laboratory-derived group equation using treadmill exercise from participant data described in section 3.3 to predict AEE was applied to data from free-living conditions (section 4.2). Predictive $Eq.4_{AEE}$ showed a mean difference of 13 $kcal.d^{-1}$ and SEE of 83 $kcal.d^{-1}$. Although individual estimates for $Eq.4_{AEE}$ ranged from -24% to 21%, values for $Eq.4_{AEE}$ were within 2% of DLW_{AEE} estimates. Predictive $Eq.4_{TEE}$ individual estimates ranged from -9.5% to 8.2% and average values were within 6% of DLW_{TEE} estimates. The wide range seen using $Eq.4_{AEE}$ is in agreement with the observation that AEE is the most variable component of TEE (Goran, 1993). However, when all components of EE were analysed together (TEE) these reported differences were reduced. These results suggested that $Eq.4_{AEE}$ provides an acceptable estimate of TEE in children under free-living conditions on a group level. However, individual estimates have to be carefully considered. Group equations have been shown to increase the error of estimating EE and it is specific to the activity being performed during the calibration process. In addition, the regression approach depends strongly on how well the method controls for inter-individual variability. Our design, supported by the arguments discussed in section 4.2.4.2.6, help to explain, at least partially, the findings verified in the present study. Provided that the limitations are recognised and the errors in estimating AEE can be tolerated, group equations are an interesting possibility, mainly because individual calibration is not required.

The precision of group equations in the prediction of AEE (or TEE) are limited because of the large variability within- and between-participant identified in physical activity patterns in children. The main characteristic of physical activity in children is the absence of a standard behaviour or *pattern.* Independent of the consistency of any day-by-day routine, children are commonly able to fidget, jump, run or play anywhere. Changing the intensity of any of these activities is a function of the influence of peers, self-motivation or state of humour.

Accelerometers are a widely used tool for movement detection and the quantification of physical activity pattern. In section 4.2.4.2.7, the applicability of the characteristics of accelerometers was discussed. The widely utilised MVPA to quantify and qualify physical activity behaviours using accelerometers is primarily dependent on the determination of cut-off points for physical activity intensity, activities below 3 METs, between 3 and 6 METs, and above 6 METs. These cut-offs have been established using a variety of activities and calibration procedures, with different brands of accelerometer showing different cut-off points. However, strategies to accurately establish these cut-offs are not widely accepted. Independent of the approach of choice, the accuracy will be directly dependent on the type of activities used for calibration (Rowlands, 2007), and even in a standardised activity, individual differences in accelerometer counts are considerable (Ekelund, Aman, & Westerterp, 2003). Comparability among studies is limited as different cut-offs may have been developed using a limited sample and potentially using very specific calibration routines with a limited range of movements. It would be extremely useful to the research community if approaches were implemented to facilitate meaningful between-study comparison of accelerometer output. One approach may be the development of standards for accelerometry output by a panel of experts on behalf of a number of International organisations and their affiliates, including health organisations. An ideal scenario might be for such standards to govern the practices of accelerometer manufacturers and devices be given a warranty based on meeting industry and research standards. Adherence to specified standards in the use and subsequent analysis of accelerometry data could be a pre-requisite for publication in relevant journals.

Trost et al. (2005) has outlined that accelerometers are not a ‘plug-and-play’ device and it is necessary to have in place, strong measures to control data quality from accelerometer output. We believe that it may be better to have limited but correct information about physical activity behaviours rather than more specific information which may incur large margins of error as recently reported by Cliff and Okely (2007) when comparing 2 commonly used accelerometer cut-off points for MVPA. These authors reported that the ability to accurately convert accelerometer output into more standardised outcomes such as time spent in MVPA is critical for all aspects of pediatric physical activity research, but more rigorous testing is need. We are in agreement regarding the relevance of evaluating time spent at MVPA. However, in terms of accelerometer counts, different brands utilise different acceleration/distance conversions. To facilitate comparisons between studies, from the reduction of counts per minute back to acceleration, the expression of physical activity measured by an accelerometer expressed in meters per second^2 is an interesting alternative. This was done using the Actiheart (Brage et al., 2005) and recently applied by Brage et al. (2007) to evaluate the precision of different individual calibration procedures against a reference calibration.

Another exciting topic for discussion is accelerometer data reduction. Until standardisation procedures for data reduction from accelerometers is developed and an accepted criterion identified, data reduction procedures should be fully described. We strongly believe that when researchers cut all continuous 10 min zero records during the day, without such periods of time being recorded in a “not worn time log”, this will overestimate children’s physical activity level.

When considering the relative contribution of each body segment to the total body movement assessed using a multi-system accelerometry approach, the present study showed the predominance of wrist and ankle over trunk (waist and chest). Interestingly, the waist-mounted accelerometer, the most commonly used body location in accelerometer studies, showed the smaller relative contribution among all body locations assessed. These results should be considered carefully and the approach replicated in other cohorts. Monitoring children under free-living conditions using multi-system accelerometry is valuable as it provides a more comprehensive view of children’s movement characteristics, independent of the translation of counts to any other meaningful, but less precise variables.

‘Efforts to improve the accuracy of HR TEE estimates should be ongoing, but the benefits of any additional complexity in the methodology should not detract from the fact that HR monitoring remains one of the most feasible and cost-effective techniques for assessing TEE and/or associated patterns of physical activity…’

The above quote from Livingstone and colleagues (2000) provides an interesting overview of the heart rate technique discussed in Chapter IV, and more specifically in section 4.2.4.2.8. The reliability of all HR-based techniques used to estimate TEE showed ICC values above 0.90 considering all monitored days. A separate analysis of weekdays verified similar reliability for all methods. Analyses of heart rate (section 4.2.4.2.8) and accelerometers (section 4.2.4.2.7) showed differences in TEE and MDPA between weekdays and weekends. As discussed previously, the inclusion of at least 1 weekend day in the objective measurement of children’s physical activity patterns is advisable.

We are unaware of studies using HR-based techniques to verify AEE in different time windows validated against the DLW technique in children. Significant associations between $\mathrm{DLW_{AEE}}$ and blocks 3 and 4, both expressions of the after-school time window (section 4.2.2.4.4), reinforce the results previously detailed indicating the after-school time window is an important discretionary period representative of children’s physical activity. However, the duration of the after-school time windows should be considered. Accelerometer data showed a better association with block 3, the largest after-school time window (3.5 hr), with measured TEE.

Chapter V, the last experimental study of the thesis, is divided into two parts. Firstly, an individual regression equation was validated against the DLW measurement detailed in Chapter IV. This verified minute-by-minute energy expenditure expressed as a multiple of measured resting energy value analysed throughout 7 days of monitoring, and quantified by intensity categories and compared with the standard value of 3.5 $\mathrm{ml.kg^{-1}.min^{-1}}$ equivalent to 1 MET in children. The second part analysed three of the most commonly used physical activity indices based on heart rate ‘cut-offs' together with the measured MET to verify the agreement among them to classify physical activity patterns.

Firstly we highlight, under laboratory conditions in a range of walking and running speeds, the inadequacy of the use of the standard MET in children. This traditional approach overestimates energy expenditure, with an increased difference linearly related to speed increments.

Minute-by-minute analyses of 7 days of free-living monitoring showed an average overestimation of 64 minutes per day for MVPA using the standard MET compared with the individually-measured MET. For all intensities these differences were statistically significant ($p<0.001$). It is interesting to note that the overestimation using this standard value corresponds with the actual daily recommendation for physical activity in children (Sallis & Patrick, 1994; Biddle et al., 1997; Trost, 2005). There is an urgent need for individual measurement of the resting metabolic rate to establish individual MET values. This is critical if one wants to express physical activities as multiples of resting energy values and make further comparisons with MET-based physical activity intensity categories to define activity thresholds in children.

The second part of this study (section 5.2.4.2) showed a variability of 20% in the average time spent at MVPA when comparing $\mathrm{HR} \geq 140$ and $\mathrm{HR} > {}_{50\%}\mathrm{P}\dot{V}_{O2}$. Our results compared to observations in the literature showed that $\mathrm{HR} \geq 140$ has been consistently estimating lower MVPA time than $\mathrm{HR} > {}_{50\%}\mathrm{P}\dot{V}_{O2}$. When these two PA indices are compared with individual and standard MET measured minute-by minute, statistically significant differences were verified among all of them at MPA, but no differences were verified at VPA, except between individual and standard METs. However, whether each one of the PA indices used are under- or overestimating time at MVPA is still debatable due to the lack of a gold standard.

We have shown that PA patterns, despite being based on the same principle, are not consistently comparable. These results allow us to recommend the utilisation of a more individualised index. The $\mathrm{HR}_{50\text{-}70\%}\mathrm{P}\dot{V}_{O2}$ technique was an interesting option when compared with our validated $HRPAnet_{RMR}$, despite not being the most practical for large scale application. We suggest that PA thresholds based on simple and general HR cut-offs, are more suitable for VPA assessment.

The literature has suggested that the percentage of time heart rate is above certain pre-selected thresholds should be informative if the number and length of sustained period (bouts or bursts) could be noted. Analyses of activity bouts (section 5.2.4.2) showed that sustained periods of PA are not characteristic of children's PA patterns, as previously cited in the literature. All individual children achieved bouts as short as 5 min everyday during the 7 days of monitoring at MPA and bouts greater than 10 min were only sporadically achieved.

Mean MVPA daily-time from minute-by minute analyses spent with HR $\geq$ 140 was 39 ±34 min and for HR > $_{50\%}P\dot{V}_{O2}$ was 52±32 min across all monitored days. Mean individual MVPA, considering the sum of bouts was 17.3±19 min for HR $\geq$ 140 and 19.7 ±16.6 min for HR > $_{50\%}P\dot{V}_{O2}$. These results evidenced that two-fold less time was spent at MVPA when considering daily-time from the sum of bouts, independent of the index used.

Each index used in this study classified different numbers of participants as achieving the PA target 60 $min.d^{-1}$. Considering that this recommendation is for most days of the week, the target was achieved by 5 children if using $Stand_{met}$ and for a single child if using Ind_{met} or $HR_{50\text{-}70\%}P\dot{V}_{O2}$. When using HR - 140 and 160, no one of the participants achieved the target. Analyses of the number of children who achieved the target at the weekend showed 9 to $Stand_{met}$, 5 to Ind_{met}, 4 to $HR_{50\text{-}70\%}$ $P\dot{V}O_2$ and 1 to HR - 140 and 160.

The wide variability when classifying children who meet the recommended target based on different indices is cause for great concern as routinely these indices are utilised as screening tools in pediatric or public health settings and form the basis of decision making regarding behavioural interventions. Importantly, choosing the most appropriate instrument depends not only on the validity and reliability of the measure, but what the instrument is measuring, whether it meets the intended purpose of the assessment, the resources needed, and the population group of interest (Dollman et al., on behalf of ACAORN, unpublished). Continuous effort is needed to facilitate the standardisation and dissemination of the most appropriate physical activity measurement tool.

6.2 Conclusions based on the key research questions

Chapter II – RMR in children

- There were no differences in RMR measurements due to body position (seated or lying) and equipment used for airflow collection during indirect calorimetry (face mask and mouthpiece/nose-clip) when each variable was isolated.
- Analyses of distraction (TV or no TV) in three of four combinations among body position and apparatus indicated no difference between TV viewing or rest alone.
- Significant differences were verified when analysing distraction combined with seated body position and using mouthpiece/nose-clip against distraction combined with a lying body position and using the facemask. When methodological issues are not stringently controlled the result is increased bias and variability thereby reported differences among children's RMR measurement.

Chapter III – Determinants of walking EE in children

- The SS determined using an indoor over-ground protocol is reproducible on the treadmill.
- The proposed 10 min familiarisation protocol based on self-selected speed provided sufficient exposure to achieve accommodation to the treadmill.
- Submaximal exercise showed within- and between-day reliability for $\dot{V}O_2$, gross economy and HR in children.
- The SS is an adequate exercise intensity to increase fat oxidation in children.
- The substrate oxidation pattern is reproducible in children performing submaximal exercise.
- Differences in oxygen uptake between girls and women performing submaximal exercise are dependent on the scaling method used. Differences verified in absolute $\dot{V}O_2$ values were normalised using a simple ratio with body mass.

- In adult women, fat utilisation starts to decrease at a velocity slower than the self-selected speed, whereas for girls the fat oxidation rate starts to plateau at this speed.

Chapter IV – Energy expenditure in children's free-living activities

- Determination of upper body or lower body activity and the utilisation of selected HR equations for each body extremity improved the accuracy of free-living TEE measurements in children.
- The simultaneous use of accelerometer and HR analysed using branched equations improved the accuracy of free-living TEE measurements in children.
- There is large within– and between-participant variability in MDPA from accelerometer outputs from 7 days of monitoring (~30 and 40%, respectively).
- The within-individual variability in TEE from HR techniques was ~10%. Between-participant variability increased by ~20%.
- Children were more active during weekdays than at weekends, independent of the measurement technique.
- The utilisation of a combination of weekdays and weekends (all days) showed better reliability for MDPA and TEE.
- The after-school time window was verified as a discretionary period for children's physical activity. Accelerometer analyses showed a better association between block 3, a large after-school time window (3.5 hr), and measured TEE.

Chapter V – Physical activity patterns in children

- The standard MET value is not applicable in children.
- Evaluation across a range of walking and running speeds, and using the measured and standard MET, showed that the traditional approach overestimates energy expenditure, with increased differences linearly related to speed increments.

- Minute-by minute analyses of 7 days of free-living monitoring showed an average overestimation of 64 minutes daily for MVPA using the standard MET compared with the individually-measured MET.
- Consistent with the findings of the previous chapter, children were more active during weekdays than at weekends, independent of the indices used.
- The most commonly utilised HR indices to measure physical activity intensity showed a variability of 20% in the average time spent at MVPA.
- Comparisons among these two common HR indices, individual and standard METs analysed minute-by minute, showed differences among all of them at moderate physical activity, but no differences were verified at vigorous physical activity, except between the individual and standard MET.
- There is no agreement between outputs from different indices when classifying children using the actual physical activity recommendations.

6.3 Implications for theory and practice

Consistently, reports in the literature identify that a sedentary lifestyle contributes to the progression of a range of chronic degenerative diseases. Measurement of energy expenditure and physical activity pattern in children is a challenge for all professionals involved in pediatric health and from a broader perspective, the public health fraternity responsible for the dissemination of population health messages.

Findings from the thesis have the potential to contribute to theory and practice in the following ways:

1. To allow between-study comparisons and apply as a ratio with other biological parameters, methodological issues have to be stringently controlled in the measurement of RMR in children;
2. To improve fat oxidation during walking in children, intensity can be controlled using the self-selected speed as an upper cut-off point. Walking at this intensity may facilitate increased exercise adherence and the avoidance of fatigue without loss of exercise efficiency from a substrate utilisation perspective;
3. Accurate, indirect and objective TEE measurements in children during free-living conditions are possible using a multi-system approach combining accelerometry and heart rate measurements;
4. Physical activity behaviours evaluated as moderate-to-vigorous physical activity based on the traditional MET classification should be carefully revised if precisely measured resting energy expenditure is not applied on an individual basis.

6.4 Recommendations and further research

The main focus of this thesis was on the measurement of energy expenditure and physical activity behaviours in children under free-living conditions during the school-term. It is important that further studies verify the energy expenditure of children during vacation periods as well as consider seasonal variation throughout a year. Choice of the school-term for the present study was based on the belief that the school is an important environment for children to adopt and increase health behaviours, including physical activity behaviours. There is evidence (Belli et al., (2005) that making greater investments in children's health results in better educated and more productive adults. Additionally, safeguarding health during childhood is more important than at any other age because poor health during the early years of life is more likely to permanently impair individuals over the course of the lifespan. Further, the work of Flodmark et al. (2006) on the use of interventions to prevent obesity in children and adolescents concluded that it is possible to prevent obesity during the growing years through limited, school-based programs that combine the promotion of healthy dietary habits and physical activity.

There is still no consensus regarding the relationship between physical activity and sedentary behaviours. Some believe that the substitution of physical activity for sedentary behaviours may prove to be an important individual difference that may predict shifts in energy balance (Epstein, Roemmich, Paluch, & Raynor, 2005). Others have argued that there is no consistency in the hypothesis that sedentary behaviours replace physical activity, suggesting that sedentariness is not the opposite of physical activity because it is comprised of several types of behaviour with their own determinants. More information on these determinants is needed to develop effective interventions that stimulate children and adolescents to diminish the time they spend on inactive behaviours (Van Der Horst, Paw, Twisk, & Van Mechelen, 2007).

Despite this diversity of opinion, Styne (2005) has suggested that there is no risk associated with a decrease in sedentary activity or a reasonable and sustainable increase in physical activity. Thus, even if we have difficulty in directly and substantially linking physical activity to fat mass, or another health index, the risk-benefit ratio is so high that there is no justification for not supporting an increasingly active lifestyle.

From intervention studies, it is known that parental influences, peer support, school-based physical education and length of physical activity time at school are important, changeable determinants of physical activity in youth (van der Horst, Kremers, Ferreira, Singh, Oenema, & Brug, 2007). The findings of the present thesis allow us to recommend that more attention is paid to specific periods of time during the school day, particularly in the morning, which has been shown to be the least active time of the day. Recently, Stratton et al. (2007) showed that normal-weight boys use school recess time as an opportunity to be significantly more active than normal-weight and overweight girls and overweight boys. Strategies to increase physical activity behaviours during school recess should be considered. Increasing time for school recess and giving specific periods for “meal-time” and “play-time” during these breaks would be a viable option. Another possibility would be the creation of specific school recess periods ‘play-times only’ and disassociated from the traditional recess time for meals. Most importantly there is a need to better account for the determinants of physical activity for each age group. Studies to compare strategies should use objective and validated tools to measure energy expenditure and physical activity.

We are conscious that the tools validated in this thesis may not be ‘ideal’ for large scale studies but may be viable for some. Unfortunately, there is no ‘easy way’ to objectively, consistently and accurately measure energy expenditure and physical activity behaviour. The body of knowledge in this area is increasing rapidly based on technological advances allowing the utilisation of indirect, objective and low-interference techniques under free-living conditions, as demonstrated by two techniques used in the present thesis, $\text{HRPAnet}_{\text{RMR}}$ and $\text{AH}_{\text{branched}}$.

The Actiheart is unique in the measurement of simultaneous HR and activity and the work in this thesis was the first validation study in children under free-living conditions. Despite being a very promising tool, minimising the main problem of HR monitoring techniques by using activity counts during low-intensity activities and the comprehensive software to clean up data, further studies are needed. Additional work is needed to verify the generalisation of our findings in samples with different characteristics, for example undernourished and obese children as well as children across a variety of fitness levels and physical activity behaviours from sedentary to very active.

In conclusion, the following citation synthesises the feelings of many researchers committed to a better understanding of physical activity and energy expenditure:

'While the optimum epidemiological tool for assessing both total energy expenditure and associated patterns of physical activity does not exist, those who work in the area have drawn up an ambitiously long 'wish' list of criteria for the 'ideal' method. The ideal method should be accurate, precise, objective, simple to use, robust, cause minimal intrusion into habitual physical activity patterns, socially acceptable, time-efficient and it should allow continuous and detailed recording of usual activity patterns.'

Livingstone (1997)

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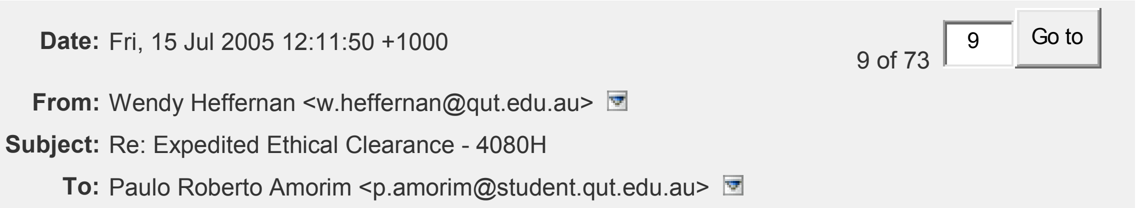
APPENDICES

Appendix 1: University Human Research Ethics Committee Approval (QUT4080H)



Date: Fri, 15 Jul 2005 12:11:50 +1000

From: Wendy Heffernan <w.heffernan@qut.edu.au>

Subject: Re: Expedited Ethical Clearance - 4080H

To: Paulo Roberto Amorim <p.amorim@student.qut.edu.au>

Dear Paulo

I write further to the response received regarding ethical clearance provided for your project, "Effects of body position, apparatus and TV viewing of resting metabolic rate (RMR) in children" (QUT Ref No 4080H).

On behalf of the Chair, University Human Research Ethics Committee (UHREC), I wish to advise that this response has addressed the additional information required by the Expedited Ethical Review Panel.

Consequently, I reconfirm my earlier advice that you are authorised to immediately commence the project.

Please do not hesitate to contact me further if you have any queries regarding this matter.

Regards
Wendy

Queensland University of Technology

Appendix 2: Participant Information Package and informed Consent (QUT4080H)

Queensland University of Technology

Participant Information Package

Project Title: Effects of position, apparatus and TV viewing on resting metabolic rate in children

Chief Investigator: Mr. Paulo Amorim (Ph.D. Candidate)

School of Human Movement Studies - Queensland University of Technology

Description

A sedentary lifestyle is a significant problem and consistent with modern society worldwide. One of the major reasons for this problem is that technological advances have allowed a reduction in energy expenditure for most individuals in habitual daily tasks. Obesity in childhood is a disorder with growing incidence, related to increase paediatric and adult morbidity and mortality. Diagnosis and subsequent treatment of childhood obesity is therefore necessary and knowledge of the individual caloric needs is essential to develop an appropriate diet and physical activity prescription. The resting metabolic rate (RMR) is the main part of total energy expenditure in children, especially when a sedentary lifestyle is dominant. Previously, it was thought that RMR was elevated in obese children, but many reports confirm today that there is no difference between obese and non-obese children. Measurement of RMR by indirect calorimetry allow measures of the amount of oxygen consumed (VO2) and carbon dioxide (CO2) produced during a specific period of time, and that values can be converted to calories and estimate the energy expenditure/day. However, that assessment, mainly for children, is very tedious and is imperative to obtain reliable results that children stay quietly during long period of time under the same conditions. This situation is stressful for many children and introduces errors and differences between many studies.

The aim of this study will be verify the variability within-test and reproducibility of RMR measurement in children through different position (seated or lay down), different apparatus (mouthpiece and nose clip or facemask), and different situation (TV viewing or no TV). Hypothetically we can reduce children anxiety that occur habitually during long time exposed to RMR measurements, reducing the measurement error, with good reproducibility, by manipulation on these three independent variables.

Subjects

The sample will comprise by 20 children (10 male and 10 female) aged between 8 and 12 years. Subjects will be recruited from the Brisbane metropolitan area through press releases in newspapers and advertisement distributed in schools. Parents or caregivers of respondents will be interviewed by telephone and subjects will be classified as eligible based on age and medical history. Exclusion criteria include individuals with known medical conditions such as diagnosed Type I or Type II diabetes mellitus or any metabolic disease. Participants who regularly take medication known to affect heart-rate or interfere with respiratory capacity or response will be excluded. Eligibility for the study will be dependent on subjects being ambulatory. Additional exclusion criteria include inability to tolerate any necessary instrument or apparatus using during testing-times. Written informed consent will be obtained from parents or caregivers of each participant before start data collection.

Experimental Protocol

On the morning of testing participants will arrive at exercise physiology laboratory (SHMS - QUT- KG) by car and will be instructed to minimise physical activity prior to arrival. Participants will have abstained from moderate and strenuous exercise in the previous 24 hours and will have a fasted state for 10 hours (water except).

Prior to RMR measurement, height and weight will be taken with the participant lightly clothed and without shoes using standard protocols. Body weight will be measured to the nearest 0.1kg on an electronic scale and height to the nearest 0.5 cm using a stadiometer.

After height and weight measurement children will be asked to complete a self-report measure of pubertal status, specific by gender.

All participants will be rested for 20 min. Each participant will be monitored periodically to ensure that he/she remains awake. Data collection will take place in a thermal-regulated environment. Respiratory gases, for RMR measurements, will be collected using a MedGraphics Cardio respiratory Diagnostic Systems. Subjects will have their RMR measured two times one week apart. The RMR will be measured in three situations: a) using mouthpiece and nose-clip (MN) or facemask (FM); b) sitting or laying position and c) TV viewing or no TV (appropriate DVD for their age and stage of development). In the first measurement session, after rest, the protocol will be: 30 min lay down: 10 min – stabilisation; 10 min using MN and 10 min using FM. Change position to seated (30 min): 10 min stabilisation; 10 min using FM; 10 min using MN. In that session, when seated, will watch DVD. In the second measurement session, after rest, the protocol will be: 30 min lay down: 10 min – stabilisation; 10 min – using FM; 10 min using MN. Change position to seated (30 min): 10 min stabilisation; 10 min using MN; 10 min using FM. At this time no watch DVD. Total time per session is around 90 min.

Expected benefits

Overall, it is the intention of the program to increase your knowledge about how much energy (in calories) you need per day. This information can help you achieve a healthy lifestyle allowing an adequate diet and physical activity prescription.

Risks

There are no risks associated with this study. The procedures involved at present study have been previously utilised in many researches and clinical settings wide world, including approved studies by QUT ethics committee, without problems.

Confidentiality

All questions, answers and results of this study will be treated with absolute confidentiality. Subjects will not to be identified in the resultant manuscripts, reports or publications and will be coded in our files.

Voluntary participation

Participation in this project is entirely voluntary. You are free to deny consent before or during the experiment. In the latter case such withdrawal of consent will be at the time you specify, and not at the end of a particular trial. You have the right to withdrawal from any experiment, and this right shall be preserved over and above the goals of this experiment.

Feedback

Feedback on your specific results and the outcomes of the project will be made available to you at the conclusion of the research project.

Questions / further information

Questions concerning the procedures and/or rationale used in this investigation are welcome at any time. Please ask for clarification of any point you feel is not explained to your satisfaction by contact with the following investigators:
Mr. Paulo Amorim: phone: 3864 5836 (email: p.amorim@student.qut.edu.au)
Professor Andrew Hills: phone: 3864 3286 (email: a.hills@qut.edu.au)
Dr. Nuala Byrne: phone: 3864 3276 (email: n.byrne@qut.edu.au)

Concerns / complaints

If you have any concerns or complaints about the ethical conduct of the project they should contact the Secretary of the Research Ethics Officer. Phone: 3864 2088 email: ethicscontact@qut.edu.au

Queensland University of Technology

Consent Form

Project title

Effects of position, apparatus and TV viewing on resting metabolic rate in children

Investigators:

Mr. Paulo Amorim, Professor Andrew Hills & Dr. Nuala Byrne

Statement of consent

The researchers conducting this project supports the principles governing the ethical conduct of research, and the protection at all times of the interests, comfort and safety of subjects. This form and the accompanying Participant Information Package *are given to you for your own protection. By signing below, you are indicating that you:*

- have read and understood the *Participant information Package* about this project;
- have had any questions answered to your satisfaction;
- understand that if you have any additional questions you can contact the research team;
- understand that you are free to withdraw at any time, without comment or penalty;
- understand that you can contact the Secretary of the Research Ethics Officer if you have concerns about the ethical conduct of the project;
- agree to participate in the project and
- give authorization to minor named *participant* participate in the project.

Participant name: ____

Signature: ____

Parent or caregiver name: ______________________________________

Signature: ____

Relationship with participant: __________________________________

Witness name: ____

Signature: ____

Date: __/__/ 2005

In case of emergency contact:

Name: ____

Telephone: ________________________**Mobile:** ___________________________

Queensland University of Technology

Participant Information Sheet (Child)

‘Effects of position, apparatus and TV viewing on resting metabolic rate (RMR) in children’

Chief Investigator: Mr. Paulo Amorim (Phone: 3864 5836)

School of Human Movement Studies - Queensland University of Technology

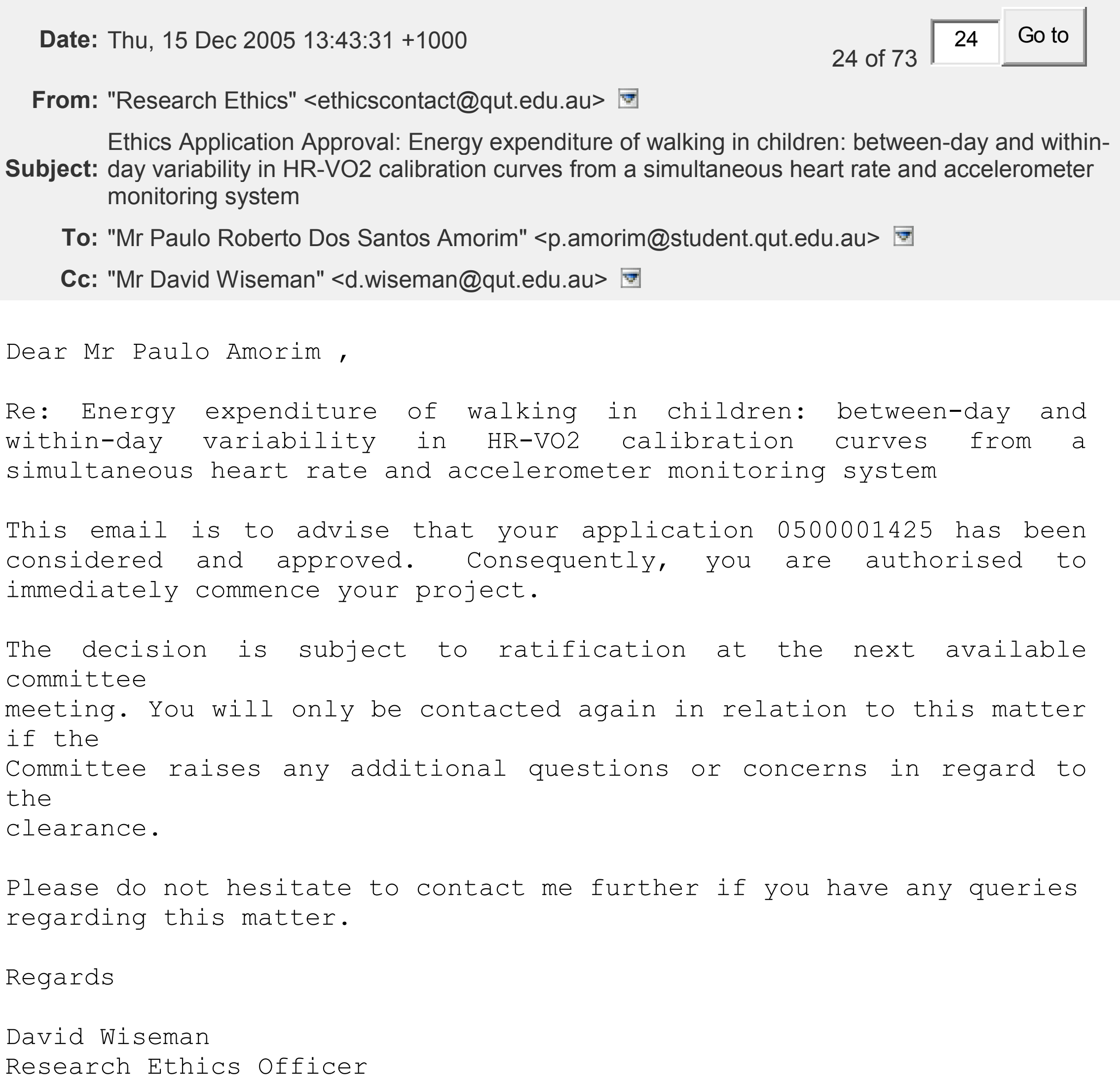
The food we eat provides us with energy for our daily life activities and also many types of nutrients. We all use the energy from food in different ways to give our body the necessary fuel to be active and involved in all of our daily activities.

The largest amount of energy needed by our bodies is the energy to keep our body going when we are resting. This is called resting metabolic rate (RMR) and it is the main part of the total energy expenditure in all children.

We need your help to measure your resting metabolic rate two times 1 week apart. On the morning of each test we will measure your height and weight and then have you rest for 20 minutes. Then we will take measurements of your breathing in either a sitting or lying position, either watching a DVD or sometimes not watching a DVD. This will take 30 minutes each test. By taking these measures we will learn more about the energy children use doing these different tasks.

Thank you very much for your help.

Appendix 3: University Human Research Ethics Committee Approval (0500001425)



Date: Thu, 15 Dec 2005 13:43:31 +1000

From: "Research Ethics" <ethicscontact@qut.edu.au>

Subject: Ethics Application Approval: Energy expenditure of walking in children: between-day and within-day variability in HR-VO2 calibration curves from a simultaneous heart rate and accelerometer monitoring system

To: "Mr Paulo Roberto Dos Santos Amorim" <p.amorim@student.qut.edu.au>

Cc: "Mr David Wiseman" <d.wiseman@qut.edu.au>

Dear Mr Paulo Amorim ,

Re: Energy expenditure of walking in children: between-day and within-day variability in HR-VO2 calibration curves from a simultaneous heart rate and accelerometer monitoring system

This email is to advise that your application 0500001425 has been considered and approved. Consequently, you are authorised to immediately commence your project.

The decision is subject to ratification at the next available committee meeting. You will only be contacted again in relation to this matter if the Committee raises any additional questions or concerns in regard to the clearance.

Please do not hesitate to contact me further if you have any queries regarding this matter.

Regards

David Wiseman
Research Ethics Officer

Appendix 4: Participant Information Package and informed Consent (0500001425)

Queensland University of Technology

Parents Information Package

Energy expenditure of walking in children: between-day and within-day variability in HR-VO_2 calibration curves from a simultaneous heart rate and accelerometer monitoring system.

Chief Investigator: Mr. Paulo Amorim (Ph.D. Candidate)

School of Human Movement Studies - Queensland University of Technology

Description

There is increasing evidence that regular physical activity contributes considerably to better physical fitness and good health of individuals. Despite being variable between people, individual heart rate and oxygen uptake tends to be linearly related throughout a wide range of aerobic exercise tasks. When this relationship is known, the exercise heart rate can be used to estimate oxygen consumption and then compute energy expenditure. The accuracy of the relationship between energy expenditure and heart rate depends on the appropriateness of an individually predetermined heart rate. There are differences between individuals and within individual in different situations under different exercise intensity levels. Therefore, it is necessary to develop an individual calibration curve for each required walking speed. Studies using the preferred pace to determine lower and faster velocity and between day and within-day variability of the calibration curves to express walking energy expenditure by heart rate and accelerometer counts, under controlled conditions in children have not been previously reported. The use of the preferred pace is useful because it reflects the speed that an individual would select during the common walking tasks of everyday life as well as during exercise. In relation to exercise prescription, knowledge of the repeatability of between-day and within-day calibration curves could to allow necessary adjustments in daily-based energy expenditure, considering the effects of diet and exercise intervention to induce weight loss.

The aim of this study is to determine the variability between-day and within-day in heart rate - oxygen consumption calibration curves in children. The main purpose is to consider how children of different age, gender, size and shape manage self-paced walking relative to energy expenditure. The energy cost of walking is an important determinant of a person’s functional capacity, physical fitness and health status.

Experimental Protocol

The experimental protocol will include three testing sessions:

Session 1: *Assessment of anthropometry, self-paced walking trials and subject familiarisation with treadmill*

Baseline data of body size and shape, and heart rate at rest will be collected. The nature of the tests will be explained and you will be familiarised with the test apparatus prior to measurement. The self-paced walking (SPW) will be determined on a level surface. by measuring the time required to walk 20 m. The field tests enable the reliability of self-paced walking to be assessed along with heart rate and accelerometry. Your individual self-paced walking speed will be replicated in the laboratory test. Walking trials will be followed by a familiarisation with the treadmill and gas analysis apparatus.

Session 2: *Treadmill test of self-paced walking and VO_2 test of cardiorespiratory function*

The laboratory test is essential to establish the difference between your energy cost of self-paced walking and lower and faster velocities. Calibration points will be obtained by simultaneous measurement of HR and VO_2 and counts RT3 for the following activities completed in sequence: standing quietly, exercising on treadmill at 2 lower paces (1.0 and 0.5 mph than SPW), SPW, faster (>0.5 mph than SPW). The protocol will involve 6 minutes at each velocity followed by 5 minutes rest between each velocity. This session include both morning and afternoon tests.

Session 3: *Treadmill test of self-paced walking and VO_2 test of cardiorespiratory function*

In this session will be repeated the same test of session 2 including a morning session only.

The Measurements:

1. **HR and accelerometry in self-paced walking at ground**

The self-paced walking will be determined on a level surface by measuring the time required to walk 20 m (5 trials), the first and the last 5 m will be discounted and the preferred walking speed will be calculated as the mean of the last 3 trials.

You will wear a HR monitor and a tri-axial accelerometer for the duration of each test. The accelerometer and HR recordings will be down loaded and the steady state HR recorded for each test. The self-paced walking speed will constitute the pace to be replicated in the subsequent laboratory test of self-paced walking.

2. **Energy cost of self-paced walking on a treadmill**

The treadmill protocol requires you to report to the University laboratory a minimum of two hours after your last food intake. You will wear lightweight, comfortable clothing and walking shoes, have abstained from strenuous exercise and consumption of caffeine, alcohol or salty foods in the previous 24 hours, and have voided a maximum of 10 minutes prior to the test. Prior to testing you will be fitted with a face-mask with two-way breathing valve. You will be fitted with a Polar heart rate monitor and a receiver to record heart rate every 5s throughout the test, and with a triaxial accelerometer. Respiratory gases will be collected throughout the test using a MOXUS Gas Analysis System.

Expected benefits

Overall, it is the intention of the program to increase your health-related fitness awareness and to develop skills to enable either weight-loss or walking for pleasure to be undertaken beyond the completion of this study. The risks are minimal, and you will be providing valuable assistance to research that could benefit many others in the future.

Risks

No measures that are to be taken will place you at any more risk than would be encountered during examinations or normal day-to-day living. In case of an emergency, expert staff and medical support facilities are located nearby (QUT Medical Support Services and Royal Brisbane Hospital).

Confidentiality

All questions, answers and results of this study will be treated with absolute confidentiality. Subjects will be identified in the resultant manuscripts, reports or publications by the use of subject codes only.

Voluntary participation

Participation in this project is entirely voluntary. You are free to deny consent before or during the experiment. In the latter case such withdrawal of consent will be at the time you specify, and not at the end of a particular trial. Your participation/ or withdrawal of consent will not influence your present and/or future involvement with Queensland University of Technology. You have the right to withdrawal from any experiment, and this right shall be preserved over and above the goals of this experiment.

Questions / further information

Questions concerning the procedures and/or rationale used in this investigation are welcome at any time. Please ask for clarification of any point you feel is not explained to your satisfaction. Your initial contact person is the investigator (Mr Paulo Amorim: phone 38645836) conducting this project.

Concerns / complaints

If you have any concerns or complaints about the ethical conduct of the project they should contact the Secretary of the University Human Research Ethics Committee on 3864 2902.

Feedback

Feedback on your specific results and the outcomes of the project will be made available to you at the conclusion of the research project.

Queensland University of Technology

Participant Information Sheet (Child)

Energy expenditure of walking in children

Chief Investigator: Mr. Paulo Amorim (Phone: 3864 5836)
School of Human Movement Studies - Queensland University of Technology

The food we eat provides us with many nutrients and energy for our daily activities. It is very important to understand how much energy we take in the form of food and how much we use up being active. A good balance is important for our growth and development. We are interested to know how much energy children use at their normal walking speed and also at lower and faster rates.

When we exercise the amount of energy we use relates to our heart rate during that task eg. walking. When we know this relationship for an individual, the exercise heart rate can be used to estimate energy expenditure during walking. Portable heart rate devices help us to make it simpler to use this technique to quantify daily energy expenditure in ‘real world’ situations.

We need your help to measure the energy cost of self-paced walking (three times on two different days). The first day will include a morning and afternoon session and the other day will only be a morning session only. On day one we will measure your height, weight, three body skinfolds and three body circumferences. In addition we will take measurements of your breathing during the following: standing quietly, exercising on a treadmill at 2 lower speeds (1.0 and 0.5 mph slower than your self-paced [normal] walking), at normal walking speed, and faster (>0.5 mph). The test will involve 6 minutes walking at each speed with 5 minutes rest between each speed. By taking these measures we will learn more about the energy children use doing these different tasks.

Thank you very much for your help.

Queensland University of Technology

Consent Form

Energy expenditure of walking in children: between-day and within-day variability in HR-VO_2 calibration curves from a simultaneous heart rate and accelerometer monitoring system.

Investigators:

Mr. Paulo Amorim, Professor Andrew Hills & Dr. Nuala Byrne

Statement of consent

The researchers conducting this project supports the principles governing the ethical conduct of research, and the protection at all times of the interests, comfort and safety of subjects. This form and the accompanying Parents Information Package *are given to you for your own protection. By signing below, you are indicating that you:*

- **have read and understood the *Parents Information Package* about this project;**
- **have had any questions answered to your satisfaction;**
- **understand that if you have any additional questions you can contact the research team;**
- **understand that you are free to withdraw at any time, without comment or penalty;**
- **understand that you can contact the Secretary of the Research Ethics Officer if you have concerns about the ethical conduct of the project;**
- **agree to participate in the project and**
- **Give authorization to minor named *participant* participate in the project.**

Participant name: ______________________________

Signature: ______________________________

Parent or caregiver name: ______________________________

Signature: ______________________________

Relationship with participant: ______________________________

Witness name: ______________________________

Signature: ______________________________

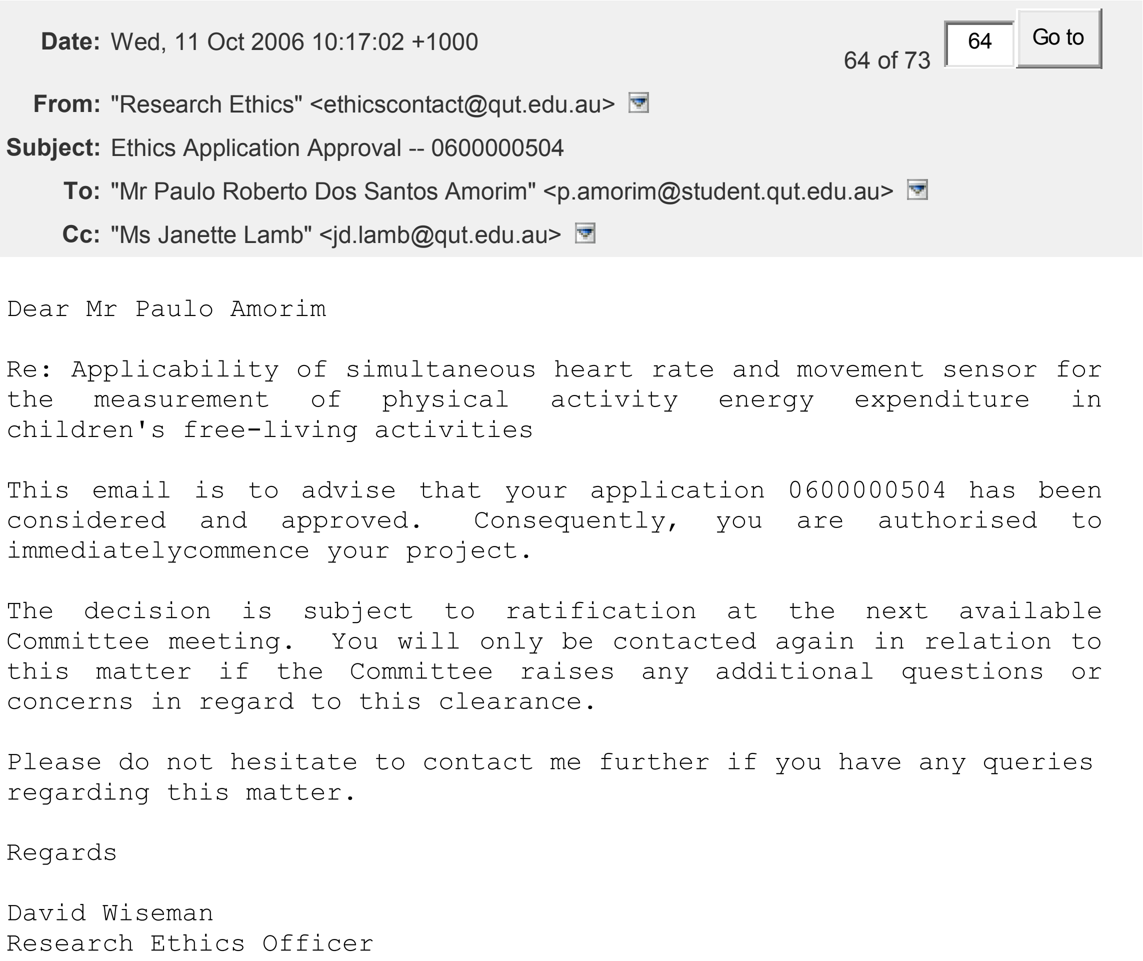
Date: __/__/ 2006

In case of emergency contact:

Name: ______________________________

Telephone: ______________ **Mobile:** ______________

Appendix 5: University Human Research Ethics Committee Approval (0600000504)



Date: Wed, 11 Oct 2006 10:17:02 +1000

From: "Research Ethics" <ethicscontact@qut.edu.au>

Subject: Ethics Application Approval -- 0600000504

To: "Mr Paulo Roberto Dos Santos Amorim" <p.amorim@student.qut.edu.au>

Cc: "Ms Janette Lamb" <jd.lamb@qut.edu.au>

Dear Mr Paulo Amorim

Re: Applicability of simultaneous heart rate and movement sensor for the measurement of physical activity energy expenditure in children's free-living activities

This email is to advise that your application 0600000504 has been considered and approved. Consequently, you are authorised to immediatelycommence your project.

The decision is subject to ratification at the next available Committee meeting. You will only be contacted again in relation to this matter if the Committee raises any additional questions or concerns in regard to this clearance.

Please do not hesitate to contact me further if you have any queries regarding this matter.

Regards

David Wiseman
Research Ethics Officer

Appendix 6: Participant Information Package and informed Consent (0600000504)

Parents Information Package

Applicability of simultaneous heart rate and movement sensor for the measurement of physical activity energy expenditure in children's free-living activities.

Chief Investigator: Mr. Paulo Amorim (Ph.D. Candidate)

School of Human Movement Studies - Queensland University of Technology

Description

This project is being undertaken as part of PhD studies for Paulo Amorim. The purpose of this project is to determine the best way to predict energy expenditure and physical activity patterns in a simple and inexpensive fashion as part of an integrated multisystems approach to population-based assessment. There is no consensus in the literature about the best method to measure energy expenditure. Amongst a large range of techniques to estimate total energy expenditure, heart rate monitoring has been one of the most widely cited. This method has been refined over time but is not without criticism. Similarly, there is no consensus about the best index to reflect physical activity patterns. The utilization of two different heart rates, one for the upper-body and another for lower-body activities simultaneously with accelerometers counts will help control for elevation in heart rate due to non-exercise reasons; these measures will be validated against doubly labeled water (DLW), a gold-standard method to measure energy expenditure. DLW is water in which both the hydrogen and the oxygen has been partly replaced for tracking purposes (i.e., labeled). The test is completely safe as the labels used are non-radioactive forms of the elements deuterium and oxygen-18 (O-18 or ^{18}O); these elements already exist in low concentrations in your body. We administer a dose of doubly-labeled water, and then track the loss of deuterium and O-18 in the participant over time through the use of regular (daily) sampling of urine. The DLW technique has several advantages for evaluating energy expenditure as it can be easily used in free-living (normal daily life). Over the past decade many studies have used the DLW technique with children. Body composition will be determined by the deuterium dilution technique, which uses the same data we have collected from DLW technique. This methodology has been shown to be an accurate and precise means of estimating children's body composition.

The research team requests your assistance because we need 30 boys and girls to participate in this study.

Participation

Your participation in this project is voluntary. You can withdraw from participation at any time during the project without comment or penalty. Your decision to participate will in no way impact upon your current or future relationship with QUT.

Your participation will involve **three visits** to QUT's Kelvin Grove facilities. Each participant will be tested to verify the individual relationship between heart rate and oxygen consumption (i.e., we calculate how your heart rate relates to the oxygen your body needs to generate energy for one specific task) for the following activities:

Session 1: – Anthropometry measures; and then carried out in sequence: supine, sitting, sitting playing computer games, sitting and completing a sub-maximal arm ergometer test. Following these measures you will perform a step-test and have a treadmill adaptation session. (Session time: 90 min)
Session 2: – Carried out in sequence: standing and walking on a treadmill at 2 slower speeds than preferred pace, at preferred pace and at a faster speed. At the end of the faster speed stage increments in treadmill will be applied until peak oxygen uptake is reached by volitional exhaustion. (Session time: 60 min).
During the measurement time (sessions 1 and 2) participants will be wearing a silicone mask which covers the face and little devices for heart rate and body movement measurement. For all treadmill activities a harness system will be used (Pictures 1 and 2).
Session 3: You will drink a small glass of labeled water and we will need samples of your urine during the next 14 days. This water is safe, has no odor or taste. For 7 days you will be fitted with four small monitors to take measurements of your heart rate and movements during your daily activities. We will provide you with all necessary information and the labeled bottles for each day of the study (Session time: 30 min).

Expected benefits

Overall, it is the intention of the study to increase health-related fitness awareness. The development of suitable indirect, objective, reproducible and inexpensive techniques to measure energy expenditure in children could be applicable to a large cohort.

Risks

No measures that are to be taken will place you at any more risk than would be encountered during examinations or normal day-to-day living. In case of an emergency, expert staff and medical support facilities are located nearby (QUT Medical Support Services and Royal Brisbane Hospital).

Confidentiality

All comments and responses are anonymous and will be treated confidentially. The names of individual persons are not required in any of the responses.

Consent to Participate

We would like to ask you to sign a written consent form (enclosed) to confirm your agreement to participate.

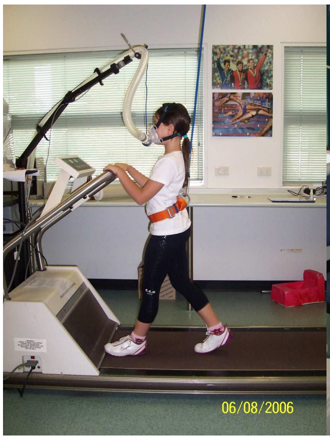
Questions / further information about the project

Please contact the researcher team members named above to have any questions answered or if you require further information about the project.

Concerns / complaints regarding the conduct of the project

QUT is committed to researcher integrity and the ethical conduct of research projects. However, if you do have any concerns or complaints about the ethical conduct of the project you may contact the QUT Research Ethics Officer on 3864 2340 or ethicscontact@qut.edu.au. The Researcher Ethics Officer is not connected with the research project and can facilitate a resolution to your concern in an impartial manner.

Picture 1



Picture 2

Participant Information Sheet (Child)

Applicability of simultaneous heart rate and movement sensor for the measurement of physical activity energy expenditure in children's free-living activities.

Chief Investigator: Mr. Paulo Amorim (Phone: 3138 6095)

School of Human Movement Studies - Queensland University of Technology

The food we eat provides us with many nutrients and energy for our daily activities. It is very important to understand how much energy we take in from food and how much we use when we are active. A good balance is important for our growth and development. We are interested in knowing how much energy children use at their free living activities.

When we exercise the amount of energy we use relates to our heart rate during that exercise eg. walking. When we know this relationship for an individual, the exercise heart rate can be used to estimate energy expenditure during our daily physical activities tasks. Heart rate monitors help us to make it simpler to measure daily energy expenditure in every day situations.

We need your help to measure the energy cost on different tasks on three days at QUT's exercise physiology lab, and in normal activities such as sitting while playing computer games, sitting and completing a sub-maximal arm ergometer test, walking on a treadmill just to cite same examples. During all these activities you will be wearing a silicone facemask. After doing these activities you will drink a small glass of labeled water and we will need samples of your urine during the next 14 days. This water is safe, has no odor or taste. Over the past decade many studies have used this water with children. We will provide you with all necessary information and the labeled bottles for each day of the study. For 7 days you will be fitted with four small monitors to take measurements of your heart rate and movements during your daily activities. It should be fun! At the end of the study, by taking these measures, we will learn more about the energy children use doing these different tasks.

Thank you very much for your help

QUT ihbi Institute of Health and Biomedical Innovation

Consent Form

Applicability of simultaneous heart rate and movement sensor for the measurement of physical activity energy expenditure in children's free-living activities.

Investigators:

Mr. Paulo Amorim, Professor Andrew Hills & Dr. Nuala Byrne

Statement of consent

By signing below, you are indicating that you:

- have read and understood the information document regarding this project;
- have had any questions answered to your satisfaction;
- understand that if you have any additional questions you can contact the research team;
- understand that you are free to withdraw at any time, without comment or penalty;
- understand that you can contact the Research Ethics Officer on 3864 2340 or ethicscontact@qut.edu.au if you have concerns about the ethical conduct of the project;
- agree to participate in the project.
- have discussed the project with your child and their requirements if participating;

Parent or caregiver name: ______________________________

Signature: ______________________________

Relationship with participant: ______________________________

Witness name: ______________________________

Signature: ______________________________

Date: __/__/ 2006

In case of emergency contact:
Name: ______________________________

Telephone: ____________________**Mobile:** ____________________

Statement of Child consent

Your parent or guardian has given their permission for you to be involved in this research project. This form is to seek your agreement to be involved.

By signing below, you are indicating that the project has been discussed with you and you agree to participate in the project.

Participant name: ______________________________

Signature: ______________________________

Date: __/__/ 2006

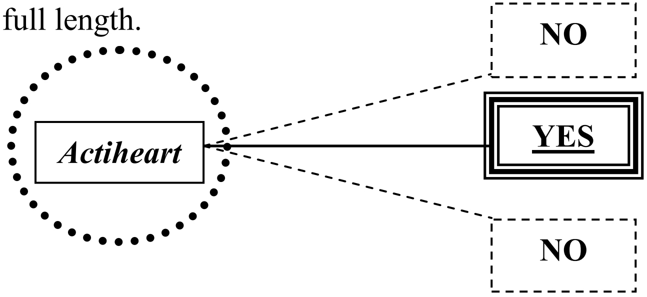
Appendix 7: Participant general instructions for free-living monitoring (0600000504)

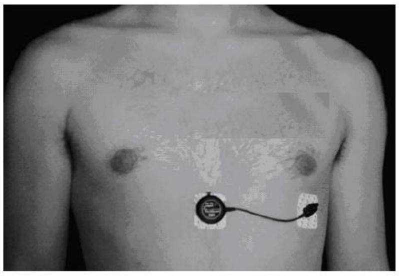
Every day:

a) Put Actibands on the waist, ankle and wrist (right side of the body with the yellow sign up for waist and ankle). Check if the ankle Actiband Velcro strap is adequately tied and cover it with a sock.
b) The times when the Actiband or Actiheart are not worn should be noted in the "log".
c) Do not collect urine sample from 1st void of the day. Urine sample must be collected from the 2nd void of the day. It is very important to be as consistent as possible with the time at which the urine sample is collected. Times should be noted on their "urine collection log" as well as on the collection bottles.
d) Keep the urine samples in a fridge.

Each three days:

a) Change Actiheart pads:
    1) Clean the skin using warm water and soap. Alcohol should not to be used as this can potentially cause skin irritation which needs to be avoided. Rub the skin with a towel or other cloth. Some redness will be seen and this is normal and should not to be cause for concern.
    2) Apply the pads to the chest. This is best achieved by placing the pad in the centre of the chest and locating the Actiheart on it. Attach the second pad to the other clip on the Actiheart and use the wire to position the second electrode. The Actiheart needs to be placed with the cable exit as near the horizontal is possible as well as stretched to its full length.





ACTIBAND & ACTIHEART - Time not worn log

Day 1

Actiheart (Chest)	Actiband (Waist)	Actiband (Wrist)	Actiband (Ankle)
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__

Day 2

Actiheart (Chest)	Actiband (Waist)	Actiband (Wrist)	Actiband (Ankle)
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__

Day 3

Actiheart (Chest)	Actiband (Waist)	Actiband (Wrist)	Actiband (Ankle)
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__

Day 4

Actiheart (Chest)	Actiband (Waist)	Actiband (Wrist)	Actiband (Ankle)
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__

Day 5

Actiheart (Chest)	Actiband (Waist)	Actiband (Wrist)	Actiband (Ankle)
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__

Day 6

Actiheart (Chest)	Actiband (Waist)	Actiband (Wrist)	Actiband (Ankle)
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__

Day 7

Actiheart (Chest)	Actiband (Waist)	Actiband (Wrist)	Actiband (Ankle)
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__
__:__ to __:__	__:__ to __:__	__:__ to __:__	__:__ to __:__

Urine Collection log

It is very important to be as consistent as possible with the urine collection time. Please times should be noted on this "log" as well as on the collection bottles.

Day	Time collected
0 (4hrs Post dose)	___:___
0 (6hrs Post dose)	___:___
1	___:___
2	___:___
3	___:___
4	___:___
5	___:___
6	___:___
7	___:___
8	___:___
9	___:___
10	___:___
11	___:___
12	___:___
13	___:___
14	___:___