Assessment of Physical Activity in Children and Adolescents

by

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Abstract

The objectives of The Identification and Prevention of Dietary and Lifestyle Induced Health Effects in Children and Infants study (child sample) and the Kenya Adolescent Physical Activity study (adolescent sample) conducted in this thesis was firstly, to assess the impact of methodological and practical decisions such as the appropriate epoch length and cutpoints to use in accelerometry studies involving children and adolescents across the physical activity continuum and the reliability of these accelerometer outcomes in predicting habitual physical activity. Secondly, the performance of uniaxial ActiTrainer accelerometry with heart rate (HR) monitoring was compared to triaxial GT3X accelerometry against indirect calorimetry during structured activities in the predominantly active Kenyan adolescent cohort. Similarly, the performance of uniaxial ActiTrainer accelerometry with HR monitoring vs. triaxial 3DNX accelerometry was compared against DLW under free living conditions in both children and adolescent cohorts. Finally, the validated uniaxial ActiTrainer was used to assess the impact of physical activity and the environment on energy expenditure and indices of adiposity in the two cohorts. The main findings of the thesis were: (a) that 15 s epoch reports significantly higher engagement in physical activity compared to a 60 s epoch in both the children and adolescents cohorts (b) choice of cutpoints significantly affected classification of physical activity and sedentary behaviour in both cohorts (c) a minimum of 6 h for 7 - 9 days in the cohort involving children and a minimum of 6 h for 4 - 5 days in the adolescents were required to reliably measure physical activity (d) triaxial accelerometry reported better predictive validity compared to uniaxial accelerometry during structured activities. In addition, HR monitoring did not improve the predictive validity of either accelerometer during structured activities (e) during free living activities, however, uniaxial and triaxial accelerometry reported comparable predictive

validity. The addition of HR monitoring improved the predictive validity of uniaxial accelerometry by approximately 4% in both cohorts (f) in both cohorts, total volume of physical activity and patterns (time engaged in light and moderate to vigorous physical activity) were significantly associated with energy expenditure (g) physical activity and sedentary behaviour were significantly related to indices of adiposity in both cohorts (h) the environment was a significant predictor of physical activity and indices of adiposity in the adolescents but not children. The findings of this thesis have important implications on strategies to standardise accelerometry field protocols and future studies on the validation of accelerometers and the association between physical activity, the environment and health.

Declaration

I hereby declare that this thesis has been composed by myself, and that the work of which it is a record has been done by myself, except where acknowledged. I also confirm that it has not been submitted in any previous application for a higher degree and that all sources of information have been acknowledged by means of references.

Robert Ojiambo Mang'eni_______________________________Date ____________________

Some of the results contained in this thesis have been published:

1. **<u>Robert Ojiambo</u>**, Hannah Budd, Kenn Konstabel, José A. Casajus, Alejandro Gonzalez-Agüero, Edwin Anjila, John J. Reilly, Chris Easton, Yannis P. Pitsiladis. Impact of methodological decisions on accelerometer outcome variables in young children. *Int J Obes.* 2011, 35: S98 - S103.

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Some of the results contained in this thesis have been presented at conferences and published as conference proceedings as follows:

1. Objective measurement of physical activity levels in Kenyan children using accelerometry. Abstract. 55th American College of Sports Medicine (ACSM) Annual Meeting, May 28 - 31, 2008, Indianapolis, USA.

2. The effect of epoch on the assessment of physical activity in Kenyan children. Abstract. 55th American College of Sports Medicine (ACSM) Annual Meeting, May 28 - 31, 2008. Indianapolis, USA,

3. The effect of epoch on assessment of physical activity in active Kenyan children. Abstract. 56th American College of Sports Medicine (ACSM) Annual Meeting, May 26 - 30, 2009 Seattle, Washington, USA.

4. The Impact of urbanisation on objectively measured physical activity levels, sedentary behaviour and indices of adiposity in Kenyan adolescents. 17th International Society for Comparatively Physical Education and Sport (ISCPES) conference 6th - 8th June, 2010 Kenyatta University, Nairobi, Kenya.

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6 Ethnic, socioeconomic and environmental determinants of health in sub-Saharan Africa. Invited Symposium. British Association of Sport and Exercise Sciences (BASES) Annual Conference *'Challenging the Dogma'* 6 - 8 September 2010. University of Glasgow, Glasgow, UK.

7 Determinants of daily energy expenditure in active Kenyan children. 58th Annual Meeting and 2nd World Congress on Exercise is Medicine of the American College of Sports Medicine (ACSM) Annual Meeting, May 31 - June 4, 2011. Denver, Colorado, USA.

Supervisor's declaration

I certify that the work reported in this thesis has been performed by Robert Ojiambo Mang'eni and that during the period of study he has fulfilled the conditions of the ordinances and regulations governing the Degree of Doctor of Philosophy.

Dr. Yannis P. Pitsiladis ___________________________________Date _________________

Dedication:

To my sons Leon & Paul, may you grow to pursue and find the truth, which should be your abiding principle all the days of your life.

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List of abbreviations

AAP – American academy of pediatrics

ADL – Activity of daily living

ADMR – Average daily metabolic rate

AEE – Activity induced energy expenditure

a.s.l – Above sea level

BM – Body mass

BMI – Body mass index

BMI z score – Body mass index standard deviation relative to WHO reference data

BMR – Basal metabolic rate

CBS – Central bureau of statistics

CHD – Coronary heart disease

CMO – Chief medical officers

CO_2 – Carbon dioxide

CPM – Average accelerometer count per minute

Cnts/15s – Accelerometer counts per 15 s

DIT – Diet induced thermogenesis

DLW – Double labelled water

FAO – Food and agricultural organisation

g – Gravitational acceleration

GOK – Government of Kenya

GPS – Global positioning system

h – Hour

HDL – High density lipoprotein

HR – Heart rate

ΔHR_{acc} – The mean difference in HR between moderate and sedentary activity i.e. (Moderate HR – Sedentary HR) using the Evenson cutpoints

ICC – Intra class correlation coefficient

IDEFICS – Identification and prevention of dietary and lifestyle induced health effects in children and infants

IOTF – International obesity task force

IREC – Institutional research ethics committee

KAPAS – Kenya adolescent physical activity study

MET – Metabolic equivalent units

min – Minutes

MOH – Ministry of health

MVPA – Moderate to vigorous physical Activity

O_2 – Oxygen

PAL – Physical activity level

ppm – parts per million

r^2 – Correlation coefficient of the model

Δr^2 – Contribution to the correlation coefficient of the accelerometer output

s – Seconds

SD – Standard deviation

SEE – Standard error of estimate

TEE – Total energy expenditure

USDHHS – United Stated department of health and human services

UGENT – University of Ghent

UGLW – University of Glasgow

UGOT – University of Gothenburg

UZAZ – University of Zaragoza

$\dot{V}O_2$– Oxygen uptake

WHO – World health organisation

y – year

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Chapter 1

1.0 General introduction and literature review

1.1 Physical activity and energy expenditure definitions and concepts:

Physical activity is defined as any bodily movement produced by skeletal muscles that result in energy expenditure (Caspersen *et al*., 1985). Sedentary behaviour on the other hand, refers to activities that do not increase energy expenditure substantially above the resting level and includes activities such as sleeping, sitting, lying down, watching television and other forms of screen based entertainment (Pate *et al*., 2008). Operationally, sedentary behaviour includes activities that involve energy expenditure at the level of 1.0 - 1.5 metabolic equivalent units (METs). One MET is the energy cost of resting quietly, often defined in terms of oxygen uptake ($\dot{V}O_2$) as 3.5 ml/kg/min. Light physical activity, which often is grouped with sedentary behaviour but is in fact a distinct activity construct, involves energy expenditure at the level of 1.6 - 2.9 METs expended during activities such as slow walking, sitting and writing, cooking and washing dishes (Pate *et al*., 2008). However, the validity of 3.5 ml/kg/min as the appropriate denominator to denote 1 MET for calculating the activity, especially when assessing the energy cost of physical activity in individuals has been questioned by several authors (Byrne *et al.,* 2005, Kozey *et al.,* 2010a, Kozey *et al.,* 2010b). The concern is that basal metabolic rate (BMR) may be influenced by ethnicity, is lower in overweight persons, declines with age, and is lower in females than in males (Vander Weg *et al.,* 2004, Henry, 2005, Kozey *et al.,* 2010a).

Thus, using a fixed BMR of 3.5 ml/kg/min to denote 1 MET may underestimate the true relationship between activity vs. rest in certain populations. It has been recommended that a correction factor be used to adjust the MET level on the basis of an estimate of individual BMR, which accounts for age, height, weight, and sex (Byrne *et al.,* 2005, Kozey et al., 2010a)

Physical activity is a complex behaviour, including sports as well as non sports activities (Caspersen *et al*., 1985). Sports are often planned, structured and repetitive, with the objective of improving or maintaining physical fitness (Caspersen *et al*., 1985), whereas non sports activities can be subdivided into different categories such as occupational, leisure time and household activities (Ward *et al*., 2005). Furthermore, physical activity includes static and dynamic activity (Westerterp, 1999b). Static activity involves static exercise and maintenance of body posture (Westerterp, 1999b). However, the impact of static exercise like weight lifting on the total level of normal daily physical activity is generally assumed to be negligible for the average subject. Similarly, the energy expenditure of maintaining body position per unit of time, is negligible, compared to the energy expenditure of dynamic activities but the time spent sitting and standing can add up to several hours per day (Westerterp, 1999b), which defines sedentary behaviour.

Physical activity is a complex, multidimensional construct which is difficult to measure (Plasqui & Westerterp, 2007). Physical activity parameters include intensity*,* frequency and duration, which together make up the total volume of activity (Corder *et al*., 2008). The intensity of physical activity is used broadly and covers a range of different phenomena, e.g., vertical acceleration of the body, heart rate (HR), or the rate of chemical energy expended over and above the basal metabolic requirements of the body (Corder *et al*., 2008). These physical activity intensity variables are expressed in relation to time (e.g., metres/second (m/s^2), beats/min) (Corder *et al*., 2008). In addition, physical activity can be expressed based on energy expenditure by combination with a measurement of BMR (Plasqui & Westerterp, 2007). As such, average total energy expenditure (TEE) is expressed in three components: BMR, diet induced thermogenesis (DIT) and activity induced energy expenditure (AEE) (Westerterp, 2003). BMR covers the energetic cost of the processes essential for life. Under most circumstances, the BMR of an individual accounts for the largest proportion of TEE and is mainly determined by fat free body mass (Westerterp, 2003). DIT on the other hand, results from the digestion, absorption and conversion of food. DIT is about 10% of the TEE in subjects consuming an average mixed diet that meets energy requirements (Westerterp, 2003). In addition, AEE is the energy expenditure associated with muscular contractions involved in body movements and maintenance of posture and is the most variable component of TEE (Westerterp, 2003). AEE is expressed as AEE = 0.9 x TEE - BMR, assuming the third component of TEE, i.e. DIT, is a constant fraction of 10% of TEE (Plasqui & Westerterp, 2007). Depending on body size and physical fitness, a 5 - 20 fold increase in metabolic rate can be sustained for a few minutes, while a healthy young adult can, if necessary, expend 5 - 8 times BMR over an 8 h working day (Bouchard *et al*., 1993).

Energy expenditure parameters associated with physical activity such as TEE which is measured using doubly labelled water (DLW) in combination with BMR measured using metabolic gas analysis are used to estimate AEE, which can be expressed using several indices such as the physical activity level (PAL) expressed as: PAL = TEE/BMR (Westerterp, 2003). The PAL normally varies from 1.2 - 2.5 (Westerterp, 1998). As a percentage of TEE, AEE varies from 5 in a subject with a minimum PAL of 1.2 to 45 - 50 in a subject with a PAL of 2.2 - 2.5 (Westerterp, 1998). In addition, AEE is usually one third of TEE when PAL is 1.75, which is the average reported level for the general population (Schultz & Schoeller, 1994, Black *et al*., 1996, Westerterp, 1999a, Westerterp, 2003). With the current knowledge of the interaction between physical activity and energy expenditure highlighted above, attempts have been made to modulate AEE to enhance the health benefits of physical activity. However, the direct effects of physical activity interventions on energy expenditure are relatively small when placed in the context of total daily energy demands (Speakman & Selman, 2003). It was suggested that exercise produces benefits in other components of the daily energy budget, thus generating a net effect on energy balance much greater than the direct energy cost of the exercise alone (Speakman & Selman, 2003). Moreover, BMR is the largest component of the daily energy budget in most human societies (Speakman & Selman, 2003). Therefore, any increase in BMR in response to exercise may potentially moderate the energy budget (Speakman & Selman, 2003) with important health implications.

1.2 Physical activity assessment:

The current focus on objective assessment of physical activity levels is motivated to achieve a better understanding of the dose response relationship between physical activity, sedentary behaviour and health outcomes (Wareham & Rennie, 1998, Celis-Morales *et al.,* 2012). Valid and reliable objective measures of physical activity are a necessity in studies designed to (a) determine the association between physical activity and health outcomes (b) document the frequency and distribution of physical activity in defined populations (c) determine the amount or dose of physical activity required to influence specific health parameters (d) identify the psychosocial and environmental factors that influence physical activity behaviour (e) evaluate the effectiveness of health promotion programmes to increase habitual physical activity in individuals, groups and communities (Wareham & Rennie, 1998, Trost, 2007). However, the complex nature of physical activity makes it difficult to accurately measure all aspects of physical activity and assess the impact on outcome parameters such as energy expenditure and body composition (Plasqui & Westerterp, 2007, Corder *et al*., 2008).

Accurate assessment of physical activity in children and adolescents remains a significant challenge (Kohl *et al*., 2000). This explains the continuous need for validated techniques for assessing habitual physical activity and sedentary behaviour not only to quantify accurately the relationship between physical activity and health (Celis-Morales *et al.,* 2012) but also to effectively monitor secular trends in physical activity and sedentary behaviour. However, the greatest obstacle to validating field methods of physical activity assessment in humans has been the lack of standardisation of protocols and an inadequate criterion to which techniques may be compared. As shown in Table 1.1, previous studies have attempted to validate subjective physical activity assessment tools (i.e. parental report and system for observing fitness instruction time (SOFIT)) as well as objective methods (i.e. accelerometry and HR monitors) against various criterion methods such as DLW and indirect calorimetry. Based on the diverse analytical approaches used in these studies as well as the variability of the physical assessment methods and the criterion used, the findings have been inconclusive and therefore, it is difficult to determine which of these assessment tools is most valid in assessing physical activity. Hence, there is a need to continuously look for the ideal physical activity and sedentary behaviour assessment tool, which should be suitable to measure physical activity and sedentary behaviour over periods long enough to be representative of normal daily life, with minimal discomfort to the subjects and be applicable to epidemiological studies (Westerterp, 1999b, Trost, 2007).

Physical activity assessment methods can be broadly divided into subjective and objective methods, which assess different aspects of physical activity and may be combined in any study (Corder *et al*., 2008). Self report instruments and motion sensing are currently the most popular methods for the assessment of physical activity in epidemiological research (Corder *et al*., 2008, Reilly *et al*., 2008). Subjective methods for assessment of physical activity include validated questionnaires, self report diaries and direct observation (Kohl *et al*., 2000, Trost, 2007). Objective measures of physical activity on the other hand include, HR monitors, accelerometers, pedometers, global positioning devices (GPS) and DLW (Kohl *et al*., 2000, Sirard & Pate 2001, Corder *et al*., 2008). However, a major limitation of physical activity research to date has been the lack of objective, practical and inexpensive tools to measure physical activity and energy expenditure on a large scale (Corder *et al*., 2008). Currently, DLW is considered the 'gold standard' for the determination of TEE and AEE, however the usefulness of DLW for large scale population based research is limited by participant burden and cost. Furthermore, DLW cannot be used to determine physical activity patterns (Plasqui & Westerterp, 2007). Other criterion methods such as direct observation are time intensive and impractical on a large scale (Trost, 2007).

Table 1.1: Validation studies of various physical activity assessment tools in children and adolescents

Study	Sample	Instrument	Criterion	Design	Analysis	Results
Murphy *et al*. 1988	213	Parental report	$\dot{V}O_2$	Indirect validation	ANOVA	Parental report associated with aerobic fitness
Rowe *et al*. 1997	173	SOFIT direct observation	HR	concurrent validation	Descriptive	Mean HR increased with increase in physical activity
Goran *et al*. 1993	30	BM, FFM, BMR HR	DLW	concurrent validation	Pearson correlation/ Multiple regression	r > 0.86 for TEE, BM, FFM and BMR 74% of TEE predicted by FFM, 7% HR and 5% BMR (R = 0.86)
Bray *et al*. 1994	40	CALTRAC accelerometer	Whole body calorimeter	concurrent validation	Pearson correlation/ Bland Altman	r > 0.8 for TEE systematic bias noted in TEE estimates
Livingstone *et al*. 1992	36	HR monitor	DLW	concurrent validation	Bland Altman	mean TEE difference -9.2 - +3.5% MJ/d
Pfeiffer *et al*. 2006	18	Actical accelerometer	$\dot{V}O_2$	concurrent validation	Pearson correlation/ percentage agreement	r = 0.89 >70% agreement

SOFIT - System for Observing Fitness Instruction Time
DLW **-** Doubly labelled water
HR **-** Heart rate
TEE **-** Total energy expenditure
FFM **-** Fat Free Mass
$\dot{V}O_2$ - Oxygen uptake
BMR - Basal metabolic Rate

1.3 Subjective methods:

Subjective methods for the assessment of physical activity include questionnaires, interviews, activity diaries and direct observation (Kohl *et al*., 2000, Corder *et al*., 2008). Direct observation is feasible in the assessment of physical activity under controlled conditions and as a validation criterion (Trost, 2007). Furthermore, direct observation is the most inexpensive and practical criterion measure of physical activity and sedentary behaviour. However, the total observation time required to attain acceptable day to day stability is not clear for most observational instruments (Kohl *et al*., 2000). Other drawbacks of direct observation include invasion of study participant privacy, the relatively high experimenter burden and the potential reactivity of the study participants, which makes direct observation unsuitable for assessment of free living activities (Kohl *et al*., 2000, Sirard & Pate, 2001, Trost, 2007).

Activity diaries have been successfully used in adolescents but not younger children who cope less well with this complex task of recording activity type, frequency and duration (Sirard & Pate, 2001). On the other hand, self administered questionnaires for the assessment of physical activity are commonly used because they are relatively cheap and easy to use in large scale studies (Slootmaker *et al.*, 2009). However, uncertainties exist about which dimension of physical activity is being assessed and the degree to which that assessment is valid (Kohl *et al.*, 2000). In addition, self report instruments for the assessment of physical activity have several important limitations. Firstly, the reported levels of physical activity are based on self perception of physical activity intensity and duration and are therefore prone to misrepresentation (Trost, 2007). Secondly, reported levels of physical activity may be skewed to conform to socially desirable standards (Prince *et al.*, 2008). Finally, self report instruments rely on ability to recall accurately and record type, intensity, frequency and duration of daily physical activity and sedentary behaviour which limits their usefulness in young children (Welk, 2002, Adams *et al.*, 2005, Trost, 2007). The accuracy of information collected by subjective instruments may therefore be biased by the opinion and perception of the participant, proxy reporter, or investigator (Slootmaker *et al.*, 2009). Given these limitations of self reported physical activity assessment tools and the growing interest in valid, reliable and accurate methods for quantifying physical activity and sedentary behaviour in children and adolescents, there is a growing interest in objective measures for the assessment of physical activity and sedentary behaviour (Trost, 2001).

1.4 Objective methods:

The complex and highly variable nature of physical activity makes it difficult to assess precisely under free living conditions (Plasqui & Westerterp, 2007). This notwithstanding, it remains of profound public health interest to accurately and reliably assess free living physical activity and sedentary behaviour in order to gain insight into the interaction between habitual physical activity levels, sedentary behaviour and health outcomes (Westerterp, 1999a). Objective methods for assessing physical activity involve the measurement of physiological or biomechanical parameters and use of the derived information to estimate physical activity outcomes, such as instantaneous and daily AEE (Plasqui & Westerterp, 2007). DLW and indirect calorimetry are considered the primary standards for assessment of physical activity in children and adolescents (Sirard & Pate, 2001, Corder *et al*., 2008).

1.4.1 Pedometry:

Pedometers are electronic devices used to estimate total distance travelled and the number of steps taken over a period of time (Sirard & Pate, 2001). They are generally cheaper than accelerometers and thus more feasible for use in large epidemiological studies (Berlin *et al*., 2006). Pedometers can be used to count steps and when the step length is known, walking distance and thus provide information about total walking activity (Rowland *et al*., 1997, Berlin *et al*., 2006). Pedometers usually consist of a horizontal spring suspended lever arm that moves with the vertical acceleration of the hips during ambulation and counts the number of times a certain acceleration threshold is exceeded (mechanical pedometers) or the number of zero crossings in the acceleration waveform (pizoelectric pedometers) and sum up these zero crossings to give an overall estimate of steps taken (Tudor-Locke *et al*., 2002). However, many models only store the total number of steps (not the time series of step frequency) with no additional information on the time over which these were accumulated (Corder *et al*., 2008). Consequently, these pedometers cannot assess intensity, duration, or frequency of activity bouts but only provide a value of total ambulatory activity (Berlin *et al*., 2006).

Newer generations of pedometers collect additional information on the time when the sensor was in motion, thus allowing more insight into physical activity behaviour (Beighle & Pangrazi, 2006). This additional information can yield estimates of $\dot{V}O_2$ and energy expenditure using estimates of stride length, sex, weight, or age (Corder *et al*., 2008). For instance, Eston *et al*. (1998) and Louie *et al*. (1999) reported modest to high correlations (r = 0.62 to 0.93) between pedometer step counts and $\dot{V}O_2$ during treadmill locomotion. Despite this high correlation in a specific context, the relationship between step frequency and energy expenditure varied between walking, running and other biomechanically different activities where r values ranged from 0.46 to 0.88 (Fogelholm *et al.,* 1998, Gardner & Poehlman, 1998, Leenders *et al.,* 2001). This notwithstanding, these findings indicate that several new generation pedometers may be suited for population based assessments of physical activity, since they are relatively inexpensive, reusable, objective and nonreactive physical activity assessments tools (Foster *et al*., 2005, Corder *et al*., 2008). Indeed, several large scale studies have successfully used pedometers for the assessment of ambulatory physical activity in adolescents (Vincent & Pangrazi, 2002, Duncan *et al*., 2006).

The relatively simple nature of pedometer output (i.e. steps/d) and the limited number of data reduction techniques used to summarize data, make these devices suitable for comparing walking levels between populations and studies (Berlin *et al*., 2006, Corder *et al*., 2008). However, pedometers suffer from several important limitations. Firstly, pedometer outputs from different pedometer brands are not comparable (Corder *et al*., 2008). Secondly, pedometer outputs may vary across age groups and therefore the data may not be comparable across different age groups due to differences in stride length (Berlin *et al*., 2006). Furthermore, the complex nature of free living physical activity, which may not necessarily be restricted to ambulatory activity, limits the applicability of pedometers in assessing free living physical activity (Corder *et al*., 2008).

1.4.2 Heart rate monitoring:

HR monitoring may be used to assess physical activity intensity and AEE in children and adolescents, both in controlled and free living environments (Trost *et al*., 2005, Plasqui & Westerterp, 2007). Early studies used universal HR intensity cutpoints to assess physical activity, irrespective of the large interindividual variation in HR (Armstrong *et al*., 1990) but using HR to estimate AEE in subjects without some form of individual calibration is problematic (Corder *et al*., 2008). HR monitoring may be used to broadly categorize group physical activity levels but lacks the specificity needed to estimate physical activity in individuals due to several limitations (Armstrong *et al*., 1990, Sirard & Pate, 2001). Firstly, the relationship between HR and instantaneous physical activity is relatively poor at low intensity activity. Secondly, a large interindividual variation in HR at different activity intensities means that individual calibration of the relationship between HR and physical activity and resultant $\dot{V}O_2$ is required (Ekelund *et al*., 2001).

One approach used to overcome these limitations is use of a flex HR point, defined as the point above which the HR and physical activity relationship becomes linear (Trost, 2007). The flex HR point is defined based on the lowest HR observed during sedentary activities (Trost, 2007) but there is no consensus regarding the definition of flex HR, which is also defined as the average of the lowest HR during exercise and the highest HR during rest (Trost, 2001, Valanou *et al*., 2006). Below the flex HR point, the HR and physical activity or HR and AEE relationship is more uncertain because HR can be raised by environmental conditions such as anxiety, stress, or increased temperature without a corresponding increase in energy expenditure (Brage *et al*., 2004). The flex HR method generally assumes that instantaneous physical activity and AEE are equal to rest below the flex point (Livingstone *et al*., 1992). However, this is unlikely to be the case at all times and this assumption introduces an underestimation into the prediction of AEE. In contrast, setting the flex point too low introduces positive bias (Corder *et al*., 2008). For these reasons, the flex HR method appears to be reasonably valid for estimating average daily energy expenditure on a group but not individual level. In addition, activity estimates using different applications of the flex HR method may not be necessarily comparable (Livingstone *et al*., 2000).

Practical difficulties associated with HR monitoring include, interference or signal loss, especially during free living conditions, although novel electrode technologies may help overcome this limitation (Corder *et al*., 2008). Moreover, HR monitoring is only useful in older children and adolescents but difficult in young children (Trost, 2001). Difficulties in children include being nervous during calibration tests in unfamiliar environments, especially when a face mask is required while measuring $\dot{V}O_2$ which can alter their HR and physical activity or HR and AEE relationship (Corder *et al*., 2008). Additionally, the sporadic nature of physical activity in young children, combined with the lag of the HR response to activity, may affect accurate physical activity assessment by HR monitoring (Trost, 2001). Novel approaches that combine HR with data from motion sensors such as accelerometers may overcome some of the limitations of the respective devices/approaches.

1.4.3 Global positioning systems (GPS):

A novel and potentially valuable tool for improving the assessment of physical activity involves the use of GPS (Maddison & Mhurchu, 2009). Twenty four GPS satellites currently orbit the earth and transmit signals to GPS receivers which determine the location, direction and speed of the receiver (Maddison & Mhurchu, 2009). This additional information has the potential to greatly improve the assessment of physical activity. Recent technological improvements have resulted in portable GPS units with adequate memory to store positional data over time, thus offering opportunities for obtaining locational information at low cost (Maddison & Mhurchu, 2009). In terms of free living activity, the most promising avenue for the application of GPS is in combination with accelerometer based measurement of physical activity (Terrier *et al*., 2000, Rodriguez *et al*., 2005). This approach potentially provides more information into the nature of activity with both location and intensity variables. Contextual information on where physical activity takes place or indeed where people are most sedentary would permit a more targeted approach to implementing physical activity initiatives and interventions (Maddison & Mhurchu, 2009). Approaches that combine accelerometry with GPS data have recently been described by Rodriguez *et al*. (2005). Therefore, one of the objectives of this thesis was to quantify active commuting to school using GPS in combination with accelerometry.

1.4.4 Combined sensors:

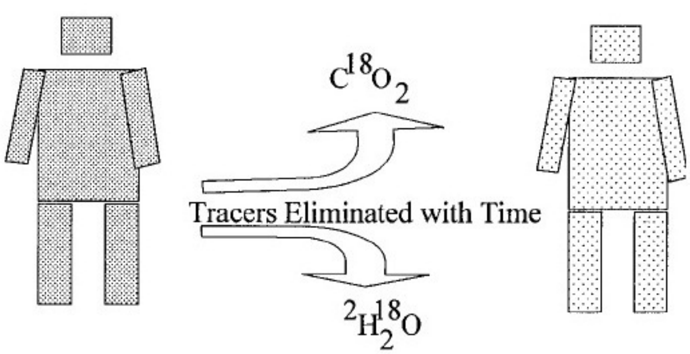
Newer sensors combining one or more physiological measures with movement sensing are continuously being developed (Chen & Basset, 2005, Corder *et al.*, 2008) in the belief that this approach will improve further the assessment of physical activity, sedentary behaviour and related outcome variables such as AEE. Prototypes including combined HR and motion sensors, combined motion and temperature sensors and multi sensor devices to determine the motion of multiple body segments are already available but prohibitively expensive for use in large epidemiological studies (Chen & Bassett, 2005). Use of multiple monitors attached to different body sites may explain more variance in activity than one alone but the extra burden and cost may not be worth the marginal increases in accuracy (Trost *et al.*, 2005). Nevertheless, many of these new methods provide comprehensive information on diverse characteristics of physical activity which may be particularly applicable to children and certain clinical populations (Chen & Basset, 2005, Bonomi & Westerterp, 2011). Thus, the predictive validity of accelerometry in combination with HR monitoring was examined in this thesis.

1.4.5 Indirect calorimetry using doubly labelled water technique:

The development of the DLW method originated from the study by Lifson *et al*. 1949. Using stable isotopes of oxygen, Lifson *et al*. administered ^{18}O labelled water to animals and showed that ^{18}O label appeared in expired CO_2. The model of Lifson assumes that the total body water pool (N) is a homogeneous compartment that remains constant during observation. Further assumptions implicit in the model are that the tracer isotopes of hydrogen and oxygen exit the body only as water and carbon dioxide and that dietary and atmospheric sources of water and oxygen do not change the background isotope levels (Lifson *et al.,* 1955). The basic mathematical equation relating carbon dioxide production to the isotope elimination rates is given as:

$rCO2 = (N/2)\ (k_O - k_H)$.

Where N is the total body water pool, k_O is the rate of disappearance of ^{18}O and k_H is the rate of ^{2}H disappearance. After a loading dose of $^{2}H_2{}^{18}O$, 5 - 20% of the tracers are lost from the body each day. The product of total body water (TBW) and the deuterium elimination rate (K_H) is a measure of the rate of water output (rH_2O). The product of TBW and the oxygen elimination rate (k_O) is a measure of the sum of water output and two times carbon dioxide output (rCO_2) (Figure 1.1). Taking the difference between these two equations and rearranging terms yields the equations for calculation of rCO_2. However, small corrections are required for differences in isotope dilution spaces and isotope fractionation (Figure 1.1).



deuterium elimination:	$r_{H2O} = TBW\ k_H$
^{18}O elimination:	$r_{H2O} + 2r_{CO2} = TBW\ k_O$
arithmetic difference:	$r_{CO2} = TBW(k_O - k_H)/2$

Figure 1.1: Indirect calorimetry using doubly labeled water (Schoeller, 1988).

The practical application of the method requires the incorporation of isotopic fractionation factors to account for fractionation (non equal equilibration) of the isotopes of water and carbon dioxide during changes in state. It is well recognized that isotopically labelled water and carbon dioxide leave the body at different rates depending on their chemical state, either gas or liquid (Schoeller, 1988, Speakman, 1997). Measured isotope fractionation factors for ^2H and ^{18}O indicate that breath water, non sweat water vapour and expired CO_2 are isotopically fractionated relative to body water. With this correction, the equation describing the model becomes:

$rCO_2 = (N/2\ f3)\ (k_O - k_H) - rH_2OG\ (f_2 - f_1)/2\ f_3$.

Where f_1 is the deuterium fractionation factor between water and water vapor, f_2 is the ^{18}O fractionation factor between water and water vapor, f_3 is the ^{18}O fractionation between water and carbon dioxide and rH_2OG is the rate of water loss via isotopically fractionated routes (Schoeller, 1988).

The isotope fractionation factors currently used are:

$f_1 = 0.941\ ^2H_2O$ (gas) / ^2H_2O (liquid)

$f_2 = 0.992\ H_2{}^{18}O$ (gas) / $H_2{}^{18}O$ (liquid)

$f_3 = 1.039\ C^{18}O_2$ (gas) / $H_2{}^{18}O$ (liquid)

The DLW method measures CO_2 production which is used to calculate energy expenditure, therefore a form of indirect calorimetry (Speakman, 1997). Heat production can be calculated by using standard indirect calorimetric relationships. This, however, requires knowledge of the metabolic fuel because heat released per litre of CO_2 produced differs by 30% between carbohydrates and lipids. This information could be obtained through continuous monitoring of respiratory gas exchange but it would defeat the non restrictive character of the DLW method (Speakman, 1997). It is more convenient, therefore, to estimate the metabolic fuel mix from dietary intake. Black *et al*. (1986) demonstrated that the respiratory quotient (RQ) is quite similar to the food quotient and that the former can therefore be predicted from the latter. The error in calculating energy expenditure from the food quotient is less than 3% in most situations, although care must be taken to account accurately for alcohol intake. In addition, if energy intake differs from energy expenditure, then some adjustment should be made to the food quotient to correct for body fat utilization or storage (Speakman & Krol, 2005). This effect on calculated heat production is not more than 5% unless the difference between intake and expenditure exceeds 20% (Black *et al*. 1986). Thus, one of the objectives of this thesis was to use the DLW method as a validation criterion against uniaxial vs. triaxial accelerometry in the assessment of free living energy expenditure in children and adolescents.

1.4.6 Indirect calorimetry using metabolic gases:

Measurements of $\dot{V}O_2$ are used to make judgments about cardiovascular function, predict endurance performance, and to estimate energy expenditure at rest and during exercise. Such measurements have been made throughout this century, progressing from Douglas bag gas collections and chemical gas analyses to integrated metabolic carts that measure $\dot{V}O_2$ and carbon dioxide ($\dot{V}CO_2$) production on a breath-by-breath basis, in addition to ventilation ($\dot{V}_E$), fraction of expired oxygen (F_EO_2), fraction of expired carbon dioxide (F_ECO_2), and heart rate (HR), do the calculations, and print the results. In spite of these developments, most metabolic measurements are still made in the laboratory setting using a limited number of exercise modes, primarily walking and running on a treadmill and pedaling a cycle ergometer (McLauglin *et al.,* 2001). Attempts to collect expired gases in the field for later analysis in the laboratory meant using cumbersome equipment that often interfered with the activities under investigation. The development of miniaturized metabolic measurement systems has allowed $\dot{V}O_2$ to be measured outside the laboratory in a natural environment (Sirard & Pate, 2001). Portable metabolic measurement systems have been used to quantify the energy cost of a wide range of activities (Bassett *et al*., 2000, Strath *et al*., 2000, Trost, 2007). Despite these advances, the equipment is still too cumbersome to use under long term free living conditions, especially in young children (Sirard & Pate, 2001). One of the objectives in this thesis was therefore, to validate uniaxial accelerometry with HR monitoring vs. triaxial accelerometry against indirect calorimetry in adolescents.

1.4.7 Accelerometry:

Motion sensors are probably the oldest tools available to measure body movement or physical activity. They have evolved from mechanical pedometers to electronic uniaxial and triaxial accelerometers (Plasqui & Westerterp, 2007). Accelerometry is now the most commonly used objective method of physical activity assessment in children and adolescents and it has greatly increased in popularity relative to other objective methods in all age groups (Rowlands, 2007). A review of physical activity measurement in preschool children reported that 63% of habitual monitoring devices used were accelerometers, mainly the ActiGraph model (Oliver *et al*., 2007). Accelerometers are electronic motion sensors that consist of pizoresistive or pizoelectric sensors (Chen & Basset, 2005, Yang & Hsu 2010, Bonomi & Westerterp, 2011). Pizoresistive accelerometers respond to accelerations by a change in resistance of silicon resistors, which is then transformed to a voltage proportional to the amplitude and frequency of the acceleration of the small mass in the sensor (Chen & Basset, 2005, van Hees *et al.,* 2010). In addition, pizoresistive accelerometers require an external power source and respond to a constant acceleration such as gravity (De Vries *et al*., 2006). Pizoelectric accelerometers on the other hand, generate an electric charge in response to a mechanical force that induces acceleration (De Vries *et al*., 2006). Pizoelectric accelerometer do not respond to constant acceleration, their major advantage is that no power supply is required, except for data storage, resulting in a considerable reduction in size and weight of the device (Chen & Bassett, 2005, Bonomi & Westerterp, 2011). Over the past decades, advances in technology have resulted in the development of small and light instruments that are able to collect data at a high frequency and store min by min data over several days or weeks (Welk *et al*., 2000b, Rothney *et al*., 2008b). Uniaxial accelerometers measure accelerations in one direction, usually in the longitudinal plane, whereas triaxial accelerometers measure accelerations in the anteroposterior, mediolateral and longitudinal direction. Most accelerometer based physical activity monitors use one or multiple pizoelectric accelerometers. A pizoelectric acceleration sensor consists of a pizoelectric element and a seismic mass, housed in an enclosure (Chen & Bassett, 2005, Bonomi & Westerterp, 2011). When the sensor undergoes acceleration, the seismic mass causes the pizoelectric element to experience deformation or compression. These conformational changes cause displaced charge to build up on one side of the sensor, which can generate a variable output voltage signal that is proportional to the applied acceleration (Chen & Bassett, 2005, Yang & Hsu 2010). Most traditional accelerometer devices only store a summary measure of the raw acceleration signal, termed a count (Corder *et al*., 2008). A count is a unit aimed to be proportional to the average overall acceleration of the human body in a specified period of time, referred to as an epoch. However, this proportionality may be challenged by the dynamic range of the accelerometer and downstream signal processing, such as frequency filtering (Figure 1.2), implemented with the intention to remove components of the signal unrelated to human movements (Tyron *et al.,* 1996, Rothney *et al*., 2008b, van Hees *et al.,* 2010). However, as traditional accelerometers are limited in memory and battery capacity to store raw signal data in gravitational (*g*) units, such data processing stages are implemented within the device itself and the process is irreversible once the count has been stored in local memory. This irreversible conversion prevents reanalysis of the raw accelerometer signal with the latest insights into data processing techniques (Figure 1.2) and limits inter brand comparison of accelerometer data.

During assessment of physical activity, a time sampling mechanism allows the capture of intensity, frequency and duration information. To ensure that the full range of human motions is captured independently, the sampling frequency should fulfil the Nyquist criterion, which specifies that the sampling frequency must be at least twice the frequency of the highest frequency of movement (Chen & Bassett, 2005). If this criterion is not met, measurements of rapid movement will be distorted. The general frequency in normal non impact physical activity of the centre of mass in humans is typically below 8 Hz (during running in the vertical direction) although the upper limit could be as high as 25 Hz in specific movements of the arms. For this reason, the sampling frequency of commercially available physical activity monitors generally ranges from 1 to 64 Hz (Chen & Bassett, 2005). This wide range in sampling frequency could be a significant determinant of device validity and hence there is a need to empirically determine the validity of several commercially available accelerometers, which is one of the objectives of this thesis.

After the data have been sampled, sensor output is filtered using a band pass filter. Band pass filtering allows frequencies between a preset low and high frequency limit to pass while all other frequencies are attenuated (Figure 1.2). The selection of an appropriate frequency response range for a bandwidth filter could be significant (Chen & Bassett, 2005, Yang & Hsu, 2010). An overly wide bandwidth would allow noises that are not physiologically related (baseline drift or the hardware, vibrations such as operating a motor vehicle, or electrical artefacts) to be included in the signals. On the other hand, a narrow bandwidth could cause incomplete data collection of all activities (Figure 1.2).

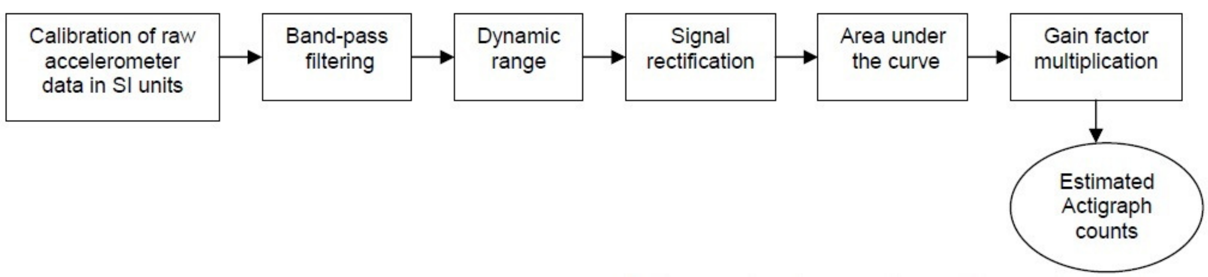


Figure 1.2: Schematic flow of accelerometer data processing (van Hees *et al.,* 2010)

Although the complete scientific description of a method should include sufficient detail of the signal processing scheme used in order to enable replication of empirical evidence, most manufacturers of accelerometer devices in use today regard the way in which the raw data is processed as proprietary information (Corder *et al*., 2008, van Hees *et al.,* 2010). Therefore, validity of accelerometer devices needs to be independently determined against physiological markers such as $\dot{V}O_2$ and energy expenditure, which is one of the primary objectives of this thesis.

There are several commercially available accelerometer based physical activity monitors, such as the Actical (Mini Mitter, Bend, OR) 3DNX^{TM} (BioTel Limited, Bristol, UK), ActiGraph (ActiGraph LLC, Pensacola, FL, USA), and RT3 (Stayhealthy, Monrovia, CA). Accelerometers may be either uniaxial or triaxial. In addition, accelerometers have different sizes and weight. There are also differences in sampling rate, sensitivity range and battery life all of which may be significant when assessing physical activity (Table 1.2). Of these accelerometers, the most studied monitor is the ActiGraph accelerometer, formerly marketed under the Computer Science and Application (CSA) and Manufacture Technology Incorporated (MTI) (Rothney *et al*., 2008b). Ideally, an accelerometer should be small, light, unobtrusive, sensitive within the right frequencies and amplitudes and able to store data over long periods of time (Plasqui & Westerterp, 2007, Bonomi & Westerterp, 2011) and thus provides information about the total amount, frequency, intensity and the duration of physical activity in daily life (Sirard & Pate 2001, Plasqui & Westerterp, 2007). The ideal physical assessment tool should be valid, affordable, objective and easy to administer, able to measure all physical activity domains, nonreactive, feasible in large studies and suitable for use in young children and adults (Table 1.2). Based on these parameters, accelerometers are indicated for the assessment sedentary behaviour and physical activity in children and adolescents. This may explain the increasing use of accelerometers in physical activity research. However, accelerometers vary in a number of ways (e.g. cost, technological sophistication, sensitivity, and output measure) (Table 1.3). Furthermore, it is anticipated that the commercial nature of these instruments will drive an even greater range of features and options in the future such as, ability to record HR and inclination, and thus, increase both the opportunities and challenges of objectively assessing physical activity (McClain & Tudor-Locke, 2009, Yang & Hsu, 2010). These monitors are based on similar accelerometry principles but they differ in their sensitivity to movement as well as in how movement is accumulated and processed within the monitor. For these reasons, it is reasonable to expect that there may be measurable differences in the validity, reliability and utility of these competing products, hence the need to determine the performance of various accelerometers in this thesis. The relative position of physical activity assessment tools, in terms of validity and feasibility may change as technology and methodologies evolve (dotted line), possibly improving validity and (or) feasibility (Figure 1.3). From figure 1.3, Eslinger & Trembly 2007, reported that direct calorimetry is the most valid physical assessment tool. However, direct calorimetry does not measure external work, for instance Webb et al., (1988) demonstrated that during cycling, energy from fuel matched heat loss plus the power measured by the cycle ergometer. During walking on the other hand, energy from fuel exceeded that which appeared as heat, meaning that work was done, this work energy did not reappear as thermal energy and therefore direct calorimetry is not more valid than indirect calorimetry for the assessment of physical activity energy expenditure. Thus, as new technologies emerge, their position on the physical activity assessment continuum must be established based on research results (Figure 1.3). Based on the assessment of validity vs. feasibility, accelerometers score comparatively well relative to other physical activity assessment tools (Figure 1.3), hence the need to validate the novel ActiTrainer, GT3X and 3DNX accelerometers in this thesis.

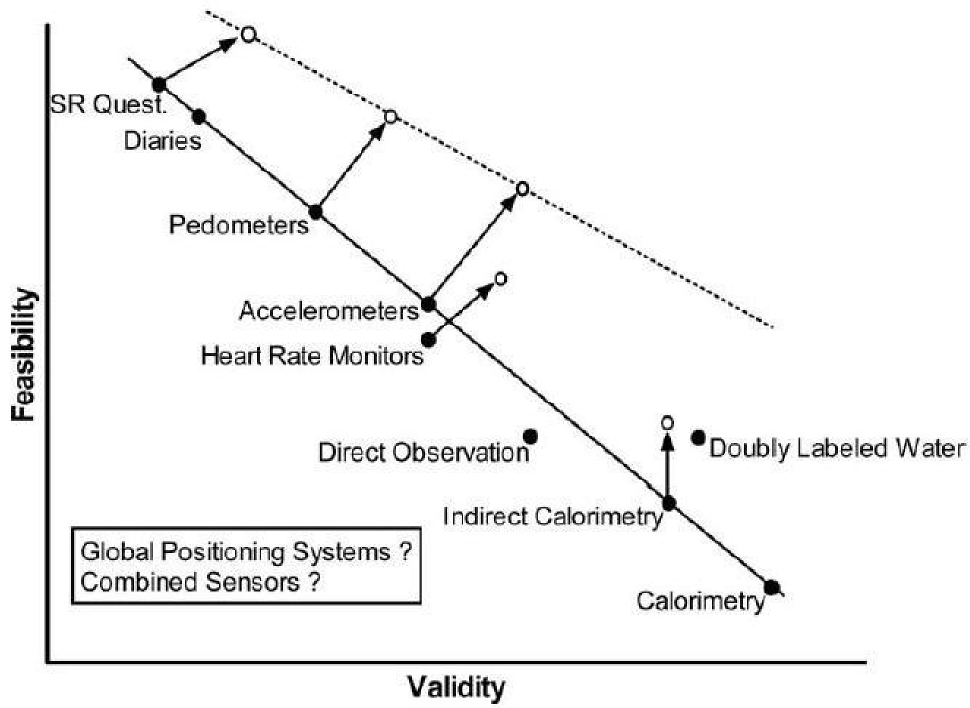


Figure 1.3: Conceptual illustration of the trade off between validity and feasibility for using a variety of physical activity measurement methods (solid line). The relative position of these measurement methods may change as technology and methodologies evolve (dotted line), possibly improving validity and (or) feasibility. As new technologies emerge, their position on the continuum must be established based on research results (e.g., GPS, novel accelerometers). SR Quest, self report questionnaire (Eslinger & Trembly 2007)

Table 1.2: Summary of key attributes of current methods of measuring physical activity in children and adolescents (Trost, 2007)

Method	Valid	Affordability	Objective	Ease of administration	Compliance	Measure PA intensity	Nonreactive*	Feasible in large studies	Suitable <10 y	Suitable >10 y
Questionnaire	√	√√√	X	√√√	√√√	√√	√√√	√√√	X	√√
Interview	√√	√	X	√√	√√	√√	√√√	√√	√	√√
Proxy report	√	√√√	X	√√√	√	√√	√√√	√√√	√√√	√
Diary	√	√√√	X	√√	X	√√√	√	√	X	√
Heart rate	√√	√√	√√√	√	√	√√	√	√	√√√	√√√
Accelerometer	√√	√	√√√	√√	√√	√√	√√√	√√	√√√	√√√
Pedometer	√√	√√√	√√√	√√	√√	X	√	√√	√√√	√√√
Observation	√√√	X	√√√	√	√√√	√√	√	√	√√√	√√√
DLW	√√√	X	√√√	√√	√√	X	√√	X	√√√	√√√

X –Poor/inappropriate √ - Acceptable √√ - Good √√√ - Excellent
* - Does not induce changes in physical activity behaviour as a result of measurement

Table 1.3: Comparison of uniaxial and triaxial accelerometer specifications

Device	GTM1	ActiTrainer	GT3X	3DNX	$Tracmor_D$	RT3
Manufacturer	ActiGraph USA	ActiGraph USA	ActiGraph USA	Biotel UK	Philips Netherlands	Stayhealthy USA
Size (mm)	53 x 51 x 22	50 x 40 x 15	38 x 37 x 18	54 x 54 x 18	32 x 32 x 5	71 x 56 x 28
Weight (g)	27	45	27	70	12.5	71.5
Number of axis	1	1	3	3	3	3
Sensor placement	waist/wrist	waist	waist/wrist	lower back	lower back	waist
Sampling rate (Hz)	30	30	0.25 - 2.5	0.2 - 20	-	0.017 - 1
Sensitivity range (G)	0.25 - 2.50	0.05 - 2.5	0.05 - 2.5	0.11 - 20	-	-
Battery life	14 days	20 days	20 days	21 days	3 weeks	30 days

- Not reported

Several reviews have concluded that accelerometers provides an objective, practical, accurate, and reliable means of quantifying amount (volume) and intensity of habitual physical activity and amount of sedentary behaviour in children and adolescents (Ward *et al*., 2005, De Vries *et al*., 2006, Rowlands, 2007, Corder *et al*., 2008, Reilly *et al*., 2008, Matthews *et al.,* 2012). Despite the initial cost and limitations for large scale research applications, accelerometers have been used in longitudinal and cross sectional studies with large samples to assess physical activity levels in children and adolescents (Corder *et al*., 2008). Advances in technology, such as the use of multiple accelerometers or their integration with other devices, such as HR monitors or GPS devices, has improved field measurement of physical activity, sedentary behaviour and AEE (Corder *et al*., 2008). However, accelerometer use is not without challenges. These include, a lack of understanding on exactly how a monitor functions, for instance, proprietary restrictions limit disclosure of which algorithms are used to convert raw acceleration into counts, how to select the most appropriate instrument, standards of monitor wear and field use, how to interpret accelerometer data and how to manipulate and analyse the vast amount of data produced by accelerometry (Trost *et al*., 2005, Ward *et al*., 2005, Matthews *et al.,* 2012). Additionally, accelerometers cannot capture certain highly static categories of activity or complex movement patterns that combine dynamic and static movements (Matthews, 2005). This is especially true of uniaxial accelerometers, but the addition of HR monitoring may improve the quality of the data collected (Corder *et al*., 2008). Moreover, standardised protocols do not exist for determining the number of monitors that participants should wear the optimal placement on the body, the optimal number of wearing days, or procedures to ensure participant compliance (Corder *et al*., 2008). In addition, it has been reported that several currently available physical activity monitors are not as sensitive to activities that are of less than moderate intensity (Hendelman *et al*., 2003) and their outputs plateau during vigorous intensity activity (Levine *et al*., 2001, Fudge *et al*., 2007). This thesis addresses some of these issues.

1.5 Methodological and practical issues in accelerometry:

Accelerometers are increasingly being used in large epidemiological studies (Riddoch *et al*., 2004). Despite the widespread use of accelerometers several methodological and practical issues remain to be resolved which have implications on all aspects of measurement, processing and interpretation of physical activity and sedentary behaviour data (Guinhouya *et al*., 2006, Corder *et al*., 2008). These include, which monitor to use and its validity, what data sampling frequency (epoch length) to use, which cutpoints to use, how many hours and days of monitoring are required to obtain valid and reliable physical activity assessment, where to place activity monitors and how to reduce the vast amount of accelerometer data generated after days of monitoring (Ward *et al*., 2005, Guinhouya *et al*., 2006, Corder *et al*., 2008). Therefore, methodological decisions are a compromise between practicality and accuracy in the assessment of physical activity and sedentary behaviour (Trost, 2007). Unfortunately, this limits the comparability between studies and explains the need to develop standardised accelerometer protocols.

1.5.1 Sampling frequency (epoch length):

Measurement of physical activity by accelerometry employs rapid sampling of accelerometer counts over a preset sampling period, or epoch (Chen & Bassett, 2005). The frequency by which the raw signal is captured and the period over which it is summarized (the epoch) are critical issues as they may ultimately affect accelerometer outcome variables (Reilly *et al*., 2008) and thus, the accuracy and reliability of physical activity and sedentary behaviour assessment (Chen & Bassett, 2005). The epoch length used should ideally be as short as possible, because data can always be reintegrated into a longer time frame but not *vice versa* (Corder *et al*., 2008). Early accelerometer studies used 1 min epoch almost exclusively to optimize the recording and storage capacity based on restrictive memory capabilities of earlier devices (Chen & Bassett, 2005). However, with greater memory capacities, decision on epoch length should be based more on research questions rather than equipment limitations (Ward *et al*., 2005).

Short epoch durations are particularly important in young children, because of the short duration of their activity bouts (Basquet *et al*., 2007). Choosing a short epoch yields higher resolution of bout durations, which may be important if physical activity is accumulated in multiple short bouts, such as in young children (Trost *et al*., 2005) or in highly active cohorts. On the other hand, the main drawback of using long epoch durations is that activities of different intensities are usually averaged to reflect an intermediate intensity and thus results in misclassification of physical activity (Chen & Bassett, 2005, Reilly *et al*., 2008). Thus, short epochs are clearly better than long epoch durations for the assessment of physical activity. Therefore, one of the objectives of this thesis was to determine the effect of epoch selection on physical activity classification in predominantly inactive children and active adolescents. For this objective, physical activity was accumulated in 1 s epoch which was subsequently integrated to 15 s epoch. The selection of 15 s epoch was based on the fact that this was the lowest epoch used in previous accelerometer calibration studies in children (Pate *et al.,* 2006). Moreover, 15 s is the lowest epoch that allows the ActiTrainer accelerometer to collect HR data, which was used in the subsequent validation studies in this thesis.

1.5.2 Physical activity and sedentary behaviour cutpoints:

The conversion of accelerometer counts into an estimate of physiological activity intensity is contentious (Puyau *et al*., 2002, Basquet *et al*., 2007). This debate makes setting physical activity and sedentary behaviour intensity thresholds difficult (Guinhouya *et al*., 2006). Usually, the current research practice involves use of a representative sample of the population of interest, in which participants wear accelerometers and another device that can accurately measure energy expenditure (i.e. portable metabolic analyser) which is used to provide interpretative count cutpoints thresholds (Corder *et al*., 2008, Reilly *et al*., 2008). These cutpoints separate movement behaviour into levels of exertion (i.e. sedentary, light, moderate and vigorous) physical activity (Corder *et al*., 2008). The current physical activity intensity thresholds for various physical activity domains derived specifically for use in children and adolescents vary widely. For example, for sedentary activity, they range from 100 - 800 CPM and for MVPA; they range from 1680 - 3200 CPM (Table 1.4). Using these different thresholds on the same data, it is possible to show that the same group of subjects is either inactive or sufficiently active, defined as accumulating at least 60 min of moderate to vigorous physical activity (MVPA) (Corder *et al*., 2008, Reilly *et al*., 2008).

To date, several ActiGraph cutpoints have been independently developed and published in the peer reviewed scientific literature (Puyau *et al*., 2002, Treuth *et al*., 2004, Pate *et al.,* 2006, Evenson *et al.,* 2008) (Table 1.4). Each equation and associated cutpoints attempts to account for the influence of growth and development on energy expenditure on accelerometer output. For example, the Freedson prediction equations include age as an independent variable in the regression model (Freedson *et al.,* 2005). The other equations/algorithms control for age related decline in BMR relative to body weight by expressing the energy cost of physical activity as multiples of BMR, METs or AEE (Puyau *et al*., 2002, Harrel *et al.,* 2005). Importantly, the methodology used to derive these cutpoints varies considerably from study to study and, this may explain the wide variability in these cutpoints. Puyau cutpoints (2002) were derived from samples with a large age range, while the Truth cutpoints (2004) were derived from a narrow age range. Similarly, the Truth cutpoints (2004) were derived from a single gender group, while the Pate (2006), Puyau (2002) and Evenson (2008) cutpoints were derived from both genders. All these factors may explain the large discrepancies in accelerometer cutpoints. Thus, one of the objectives of this thesis was to determine the effect of published physical activity cutpoints on classification of physical activity patterns in children and adolescents.

Table 1.4: Published cutpoints for children and adolescents using ActiGraph accelerometer

Author	Sample	Activities	Cutpoints
Pate *et al*. **2006**	N = 29, 3 - 5 y Mean Age: 4.4 y 16 girls, 13 boys	Structured and free living activities such as slow walk, brisk walk, jogging	MPA ≥ 420/15s
Puyau *et al*. **2002**	N = 26, 6 - 16 y Mean Age: 10.7 y 12 girls, 14 boys	Free living activities such as computer games, playing toys, aerobics, skipping, jump rope, soccer	Sed ≤800/min LPA ≥ 800/min MPA ≥ 3200/min VPA≥ 8200/min
Treuth *et al*. **2004**	N = 74, 13 - 14 y girls only	Walk, run, free living activities such as computer games, household chores, aerobics, shooting baskets.	Sed ≤100/min LPA ≥ 100/min MPA ≥3000/min VPA ≥ 5200/min
Evenson *et al*. **2008**	N = 33 Range: 5 to 8 y Mean Age:7.3 y 21 girls, 12 boys	coloring books, slow walk, stair climb, basketball, brisk walk, jumping jacks, running.	Sed ≤100/min LPA ≥ 100/min MPA ≥ 2296/min VPA ≥ 4012/min

TM - Treadmill
Sed - Sedentary activity
LPA - Light physical activity
MPA- Moderate physical activity
VPA - Vigorous physical activity

1.5.3 Monitor placement:

The relative position of the accelerometer on the body is an important consideration. Ideally, the accelerometer should be attached as close as possible to the centre of mass of the body. However, feasibility and subject burden should be carefully considered (Trost *et al*., 2005). Bouten *et al*. (1997) investigated the influence of monitor placement on accelerometer output and the prediction of energy expenditure during walking (3 - 7 km/h). Using both measured and simulated acceleration data, a total of six different body segments were evaluated: lower back, lower leg/foot, upper leg, head trunk, lower arm/hand and upper arm. Acceleration output recorded at the lower back was the best predictor of energy expenditure (r = 0.92 - 0.97), although acceleration data from all sites demonstrated moderate to strong associations with observed energy expenditure (0.52 - 0.92). Furthermore, when the authors portioned the acceleration output into the kinematic (acceleration due to body movement) and gravitational component (the acceleration due to gravitational pull of the earth), the influence of the gravitational component was strongest on the limb placements, supporting the utility of trunk placements, either on the lower back or hip (Bouten *et al*., 1997) and arguing against the use of the wrist or ankle. Nilsson *et al*. (2002) examined whether placement of a uniaxial accelerometer on the hip or lower back would affect assessment of physical activity in 16 free living 7 y old children. Over the 4 d monitoring period, no significant difference emerged between two monitor placements in total counts per min. However, when counts were classified as moderate or vigorous intensity, the hip placement resulted in higher estimates of moderate physical activity, although the difference was significant only when counts were accumulated in 5 s epoch and not in 60 s epoch. Furthermore, there was no difference between hip and back placements for estimated time in vigorous and very vigorous physical activity. Yngve *et al.* (2003) examined whether monitor placement on the hip or lower back could influence the assessment of treadmill and overland walking and running in healthy adults. Compared to the activity counts from the monitor placed on the hip, the counts recorded on the back were significantly lower during normal and fast walking and significantly higher during jogging. The magnitude of these differences, however were quite small and of questionable practical significance. Welk *et al*. (2000b) examined the issue of monitor placement using three different models of accelerometers (ActiGraph, BioTrainer, TriTrac R3D). 42 adults completed three 6 min bouts of walking with the monitor positioned in three different locations on the right hip: 1) anterior axillary line (iliac crest), 2) midaxillary line and 3) the posterior axillary line. No significant placement effects were observed on either the BioTrainer or TriTrac-R3D. However, small but statistically significant differences were observed for the ActiGraph device, with significantly higher counts recorded for the midaxillary line placement than either anterior or posterior axillary line. The practical significance of these findings is uncertain, given that it is unlikely that the average study participant would wear their accelerometer on exactly the same site on each monitoring day. Studies comparing different placement options indicate that accelerometers are best placed on the hip or lower back (Trost *et al*., 2005). The right side may be most convenient because most people are right handed (Corder *et al*., 2008). Therefore, it is recommended that one side be consistently used to standardise accelerometer monitor placement protocols (Ward *et al*., 2005). However, the propensity for waist mounted accelerometers to misclassify static light to moderate intensity activities such as folding laundry and sweeping as sedentary remains a legitimate concern. Combining accelerometry with other monitoring devices such as thigh mounted inclinometers may be a viable solution to this problem (Trost *et al.,* 2011).

1.5.4 Monitoring time:

Accelerometer monitoring for 7 consecutive days seems reasonable when assessing habitual physical activity and sedentary behaviour. However, as protocol adherence tends to decrease with days of wear (Trost, 2007, Corder *et al*., 2008, Baranowski *et al.,* 2008), it may be more feasible to opt for 4 full days with at least 1 weekend day (Trost, 2007, Corder *et al*., 2008) as typically the case in large studies (Riddoch *et al*., 2004). There is some evidence that between 4 and 9 full days of monitoring including 2 weekend days are required for a reliable estimate of physical activity behaviour in adolescents (Trost *et al*., 2005). The compromise between feasibility and accuracy was demonstrated in a large study of 11 y old children that examined the number of days of monitoring required to achieve reliability coefficients of 0.7, 0.8 and 0.9 (Mattocks *et al*., 2008a). 3 days of monitoring were required to achieve a coefficient of 0.7, irrespective of whether a valid day was counted as 420 or 600 min/day. 5 days were required for a coefficient of 0.8 and 11 days were needed to achieve a reliability coefficient of 0.9. In addition, the age of the study participants also influences reliability estimates, with younger children having less day to day variability than older children. For instance, Trost *et al*. (2000) reported that 4 - 5 days of monitoring were required to achieve a reliability coefficient of 0.8 in young children but between 8 and 9 days may be required for adolescents. Furthermore, seasonality due to school terms, school holidays and climate, is also another important factor to consider (Corder *et al*., 2008). For all methods of measurement, seasonal variation means that a single measurement of a week may not adequately reflect the subject's habitual physical activity because there may be considerable seasonal intraindividual variation (Baranowski *et al.,* 2008). However, it has been suggested that among adults at least 3 - 5 days of monitoring are required to estimate habitual physical activity (Trost *et al*., 2005). With children and adolescents on the other hand, studies have consistently shown that the number of monitoring days ranges from 4 and 9. Because of the differences between week and weekend days and the balance between feasibility and validity, it is recommended that as a minimum, studies in both children and adolescents aim for at least 4 full days of monitoring including one weekend day (Corder *et al*., 2008, Matthews *et al.,* 2012). Other legitimate considerations when determining period of monitoring in paediatric, adolescents and adult studies are, the study population of interest, budgetary considerations and the specific research questions being addressed by a given study (Trost *et al*., 2005, Ward *et al*., 2005).

1.5.5 Valid day:

Among the most important practical decisions in accelerometer studies is determination of what constitutes a day (i.e. should it be the waking day, or time periods such as 12, 18, or 24 h?) and what percentage of a day must be measured for an individual to have sufficient information to call this period a complete day (Ward *et al*., 2005, Corder *et al*., 2008). A day varies for individuals in different age groups and also may vary depending on whether the physical activity and sedentary behaviour are being measured on a weekday or a weekend day (Catellier *et al*., 2005, Màsse *et al*., 2005). Alternatively, a day can be defined by the 70/80 rule that is, the period during which at least 70% of the study population has recorded accelerometer data and 80% of that observed period constitutes a minimum day for inclusion in data analysis (Catellier *et al*., 2005). Currently, there is no single accepted criterion for the identification of how much wear time is necessary to constitute a valid day of measurement (Corder *et al*., 2008, Baranowski *et* *al.,* 2008, Matthews *et al.,* 2012). Thus, several approaches are used to address validity issues in physical activity and sedentary behaviour monitoring. These includes, the use of the Spearman Brown prophecy formula and computation of Intra Class Coefficients (ICC) to estimate the number of days or repeated observations needed to obtain a specified level of reliability in a given sample (Trost, 2007, Baranowski *et al.,* 2008). These approaches attempt to account for diurnal/seasonal variation in physical activity and sedentary behaviour and also differences in hours of monitoring within the study population and thus better approximate the assessment of physical activity and sedentary behaviour. Therefore, one of the objectives of this thesis was to determine the optimal monitoring period in children and adolescents.

1.5.6 Accelerometer data reduction:

One of the most challenging aspects of using accelerometers to measure physical activity and sedentary behaviour is managing and understanding the vast amount of data collected (Ward *et al*., 2005). With recommendations of multiple days of monitoring and with sampling epochs that may be as low as 1 s, the volume of data created from accelerometer measurement may be overwhelming (Ward *et al*., 2005). Therefore, making decisions about how data will be edited and analysed even before data collection begins and stating these 'decision rules' clearly in published articles will facilitate attempts to develop universal accelerometer protocols and thus allow for comparison between studies (Ward *et al*., 2005). Accelerometers record data continuously irrespective of whether the monitor is worn or not. Therefore, non wear time needs to be identified either using activity diaries, assumption of sleeping time or identifying data segments of continuous inactivity surpassing a length beyond which it is deemed unlikely that the monitor could have been worn, e.g. 10 - 60 min (Treuth *et al*., 2003, Corder *et al*., 2008).

Other strategies of dealing with missing data include exclusion of the time period (equivalent to imputation with the average of remaining data for an individual) to replacement by time of day specific values estimated from the remaining data on an individual or group level and various averaging schemes using scaling for weekdays and weekend days (Catellier *et al*., 2005). As a result of this diversity in accelerometer data editing and other analytical approaches, comparability between studies is often difficult (Rowlands, 2007). Consequently, it has been suggested that accelerometers should be worn for 24 h/d (Rowlands, 2007, Matthews *et al.,* 2012) but this may not be practical due to discomfort during sleep, for example from monitors positioned around the waist (Ward *et al*., 2005). Furthermore, it is important that volunteers are encouraged to follow the specified protocol, because if volunteers are instructed to wear monitors for 24 h/d but some remove the monitor at night, the data may not be comparable if expressed as a mean daily value (Ward *et al*., 2005). Thus, subject compliance can have dramatic effects on the estimates of physical activity and sedentary behaviour (Ward *et al*., 2005, Corder *et al*., 2007b). Therefore, several strategies to promote protocol adherence have been proposed to achieve 24 h monitoring (Ward *et al*., 2005, Trost, 2007b).

1.5.7 Accelerometer validation:

Accelerometry data are usually expressed as 'movement counts', an arbitrary value that is often not comparable between monitor brands (Reilly *et al*., 2008). It is therefore necessary to convert accelerometer outputs into meaningful physiological parameters such as energy expenditure or $\dot{V}O_2$ associated with specific levels of physical exertion (Corder *et al*., 2008). Energy expenditure is a physiological consequence of physical activity and is directly linked to health and disease prevention (Jakicic & Otto, 2005). Therefore, DLW and indirect calorimetry used as criterion measures for energy expenditure can also be used as criterion measures for physical activity assessment. However, it should be noted that energy expenditure and physical activity are distinct constructs, since a significant portion of energy is expended while inactive (i.e. BMR and DIT) which may limit attempts to validate physical activity measures against energy expenditure (Sirard & Pate, 2001). This notwithstanding, DLW is an excellent method to measure TEE in unrestrained humans in their normal surroundings over a time period of 1 - 4 weeks (Speakman, 1997). Thus, measurement of AEE with DLW has become broadly accepted as the reference method for validation of accelerometers. However, accelerometer output contribution to the prediction of energy expenditure (Δr^2) is highly variable in different studies and this may be due to different device specifications (uniaxial vs. triaxial) and the inherent variability of the study populations, for instance the Mâsse et al. (2004) study used a uniaxial accelerometer to assess AEE in 136 African American and Hispanic women with low reported Δr^2 of 5%. On the other hand, Plasqui et al. (2005) examined the predictive validity of the triaxial Tracmor accelerometer in 29 Dutch subjects (male and female) aged 18 - 40 y and reported Δr^2 of 33% (Table 1.5). This highly variable Δr^2 between accelerometer validation studies could be due to the inherent differences in the validity of the accelerometer devices used, the difference in the study populations, gender, age range and activity profiles (accelerometer counts/day (Cnts/d ranging from (83 - 2626 kilocounts/d) of the subjects under study. To date, of the numerous accelerometer validation studies involving DLW as the criterion, the Tracmor (Philips Research, Eindhoven, Netherlands) triaxial accelerometer has produced the best predictive validity based on the reported Δr^2 (Table 1.5). However, most accelerometer validation studies have been done in Western adult subjects who are inherently physically inactive. One of the objectives of this thesis was to determine the predictive validity of the ActiTrainer uniaxial accelerometer (ActiGraph LLC, Pensacola, FL, USA) and the 3DNX triaxial accelerometer (BioTel Limited, Bristol, UK) frequently used in studies against DLW in very young European children and African adolescents, two cohorts with greatly different physical activity levels.

Table 1.5: Accelerometer validation studies against DLW

Device	Author	n	age	Dependent	Independent	r^2	Δr^2
ActiGraph	Mâsse *et al*. 2004	136	50±7	AEE	BM Cnt/d	9%	5%
Tracmor	Plasqui *et al*. 2005	29	18-40	AEE	Age, BM, Cnts/d, H	81%	33%
3DNX	Carter *et al*. 2008	37	13-30	TEE	H, Cnts/d	73%	27%
$Tracmor_D$	Bonomi *et al* 2010	30	41±11	AEE	BM, Cnts/d	46%	16%

TEE - Total energy expenditure
AEE -Activity induced energy expenditure
BM - Body mass
H - Height
Cnts/d -Counts per day

r^2 - Correlation coefficient of the model
Δr^2 - Contribution to the correlation coefficient of the accelerometer output

It is envisaged that multiple accelerometers will be required to produce the highest accuracy in terms of predicting AEE (Bonomi & Westerterp, 2011). However, the disadvantage of this approach is the discomfort to the subject and the potential to alter the physical activity and sedentary behaviour being assessed (Westerterp, 1999b, Strath *et al*., 2005, Trost, 2005). Nevertheless, it has been proposed that using accelerometer data in combination with HR data can significantly improve the prediction of energy expenditure (Strath *et al*., 2000, Strath *et al*., 2001). For example, the addition of HR to motion sensing increased the prediction of scaled $\dot{V}O_2$ in children (Eston *et al*., 1998) and hence the efficacy of using a combined HR and motion sensor approach to estimate free living activity in the two cohorts of children and adolescents was also explored in this thesis.

1.6: Physical activity and health:

Physical activity has consistently been associated with improved physiological functioning and lower disease risk according to observations drawn from controlled experimental trials and population based epidemiological studies (Blair *et al*., 2004). There is sufficient scientific evidence to conclude that physical activity has beneficial effects on adiposity levels, blood pressure, plasma lipid and lipoproteins levels and non traditional cardiovascular risk factors (inflammatory markers, endothelial function and heart rate variability) in adolescents and adults. Moreover, physical activity has beneficial effects on several components of mental health (self concept, anxiety and depression) (Strong *et al.,* 2005, Haskell *et al.,* 2007, Warburton *et al.,* 2010, CMO, 2011). The benefits of regular physical activity have been clearly set out across the life course. In particular, for adults, doing 30 min of at least moderate intensity physical activity on at least 5 days a week helps to prevent and manage over 20 chronic conditions including coronary heart disease, stroke, type 2 diabetes, cancer, obesity, mental health problems and musculoskeletal conditions (WHO, 2010). Furthermore, there is a clear relationship between the amount of physical activity people do and all cause mortality (WHO, 2010). On the other hand, spending large amounts of time being sedentary may increase the risk of some health outcomes, even among people who are active at the recommended levels (Ekelund *et al.,* 2006, Leblanc *et al.,* 2012, Santos *et al.,* 2012, Wagner *et al.,* 2012).

Physical inactivity is responsible for 6% of deaths globally - around 3.2 million deaths per year, including 2.6 million in low and middle income countries and 670,000 of these deaths are premature (WHO, 2011). A recent analysis indicated that reaching the recommended minimum level of physical activity (at least 30 min/d) compared with no activity was found to lead to a reduction in all cause mortality by 19% and this rose to 24% if an h a day was spent in physical activity (Woodcock *et al.,* 2011). Furthermore, there is a 31% lower risk for all cause mortality in active individuals (Warburton *et al.,* 2010). This demonstrates a positive dose response - in other words, that the benefits of physical activity increase as the amount of the activity increases. In adults, the improvements in physical activity are especially pronounced for high risk individuals, for example, those who are obese or have high blood pressure (hypertensive) (Warbuton *et al.,* 2010). Research has also shown that being physically active daily will reduce the chances of mortality associated with cardiovascular disease: 30 min of moderate intensity exercise on most days of the week, equivalent to 4.2 MJ (1000 kcal) a week, was enough to reduce cardiovascular related mortality (Paffenbarger *et al.,* 1986).

Physical activity has beneficial effects on blood glucose levels. Gill and Cooper (2008) reviewed 20 longitudinal cohort studies and revealed that regular physical activity substantially reduces risk of type 2 diabetes. Adjustment for differences in BMI between active and inactive adult groups attenuates the magnitude of risk reduction but even after adjustment, a high level of physical activity was associated with a 20 - 30% reduction in diabetes risk. The data indicated that protection from diabetes can be conferred by a range of activities of moderate or vigorous intensity and that regular light intensity activity may also be sufficient, although the data for this was less consistent. The risk reduction associated with increased physical activity appears to be greatest in those at increased baseline risk of the disease, such as the obese, those with a positive family history and those with impaired glucose regulation (Gill & Cooper, 2008). Furthermore, a clinical trial of ≥8 weeks duration on HbA_{1c} (A_{1c}) and body weight in people with type 2 diabetes, demonstrated that post intervention, A_{1c} was significantly lower in those who exercised than controls (7.65 vs. 8.31%, weighted mean difference -0.66%,). In contrast, post intervention body weight did not differ between the exercise and control groups. Meta regression confirmed that the beneficial effect of exercise on HbA_{1c} was independent of any effect on body weight. Therefore, structured exercise programmes had a statistically and clinically significant beneficial effect on glycemic control and this effect was not primarily mediated by weight loss (Sigal *et al*., 2006, Yates *et al.,* 2007, Umpierre *et al.,* 2011). A subsequent meta-analysis (Boule´ *et al.,* 2003) showed that exercise intensity predicted post intervention weighted mean difference in A1C (r = - 0.91, P = 0.002) to a larger extent than exercise volume (r = -0.46, P = 0.26). These results provide support for encouraging type 2 diabetic individuals who are already exercising at moderate intensity to consider increasing the intensity of their exercise in order to obtain additional benefits in both aerobic fitness and glycemic control. In addition, Fulton-Kehoe (2001) carried out a case-control study among Hispanics in Colorado, and showed a 40% decrease in the odds ratio of being diabetic in the most active third of the population, whether leisure time or occupational physical activity was measured. A study of middle-aged Finnish adults showed that at least moderate intensity occupational activity, active commuting or leisure time physical activity were each associated with reduced diabetes incidence (Hu *et al.,* 2005). The Finnish Diabetes Prevention Study (2001) was a randomised controlled trial of 522 people with impaired glucose tolerance (IGT). The intervention included intensive nutritional counselling and some endurance exercise advice. The aims were to reduce weight by about 5% (achieved by 43% intervention, 13% controls), to reduce total fat intake to around 30% or less (47% intervention, 26% controls), and to achieve the moderate physical activity recommendations of 30 min/d (increased physical activity 36% intervention, 16% controls). Intervention subjects lost around 4.2 kg (compared with 0.8 kg in controls) at 12 months, and also showed greater reductions in waist circumference and in blood pressure than controls. Diabetes incidence was reduced by 58% more among the intervention group than controls, and was related to the amount of lifestyle change. It was concluded that for every 22 people with IGT who received the intervention, one more case of diabetes might be prevented. Furthermore, the Diabetes Prevention Project (DPP group, 2002) was a multi-centre randomised controlled trial in the USA with 3234 adults with IGT. There were three trial arms: an intensive 16 session lifestyle intervention, a usual care arm, and a pharmacological arm (Metformin). The behavioural outcomes showed that 74% achieved their physical activity goal at one year, and also achieved their weight loss goal of around 7% (weight loss of about 7 kg). There was a 58% reduction in the incidence of diabetes in the intensive lifestyle intervention group and a 31% reduction in the Metformin group, compared with controls. Given the strength of the evidence, the study was stopped early, with the behavioural intervention significantly more effective in preventing diabetes than Metformin. These trials provide evidence that diabetes can be prevented in those at high risk by lifestyle intervention.

The influence of relatively extensive physical exercise on the composition and concentration of lipoproteins was clearly exemplified in a large US study (Kraus *et al*., 2002) in which overweight men and women were divided into four groups and monitored for eight months, one control group and three exercise groups. Group A exercised (ergometer cycling, jogging) with an energy expenditure that corresponded to 32 km jogging/week at 65 - 80% of $VO_{2\ max}$, group B exercised at the same intensity but for a shorter distance (corresponding to an energy expenditure of 19 km jogging/week), while group C underwent the same amount of exercise as group B but at a lower intensity (corresponding to 40 - 55% of $VO_{2\ max}$). After 8 months of exercise, the concentration of HDL cholesterol increased only in group A (+ 9%), together with several other beneficial lipoprotein changes. Some changes were also noted in groups B and C (primarily an increase in the size of the LDL and VLDL particles) but to a much lower extent (Kraus *et al.,* 2002). Another cross sectional study was conducted on a representative sample (n = 3110) of 12 - 19 y old American adolescents that measured cardiorespiratory fitness using a submaximal treadmill test. The results indicated that unfit girls, defined as the lowest 20% fit, were 1.89 (95% confidence interval: 1.12 - 3.17) times more likely to have hypercholesterolemia and 1.03 (0.74 - 1.43) times more likely to have a low HDL cholesterol compared to moderately and highly fit girls. Unfit boys were 3.68 (2.55 - 5.31) times more likely to have hypercholesterolemia and 1.25 (0.79 - 1.95) times more likely to have a low HDL cholesterol compared to moderately and highly fit boys (Carnethon *et al.,* 2005).

Several studies indicate a relationship between physical activity and cardiovascular fitness. Physical activity causes a reduction of around 3 mm Hg for systolic blood pressure (BP) and 2 mm Hg for diastolic BP in hypertensive patients. These reductions are particularly evident for moderate levels of physical activity, including walking (Fagard, 2001, Kelly *et al.,* 2006). In addition, aerobic based interventions also result in significant reductions (~6% to 11%) in diastolic blood pressure (Dasgupta *et al.,* 2006). Regular exercise constitutes strong protection against the increased risk of cardiac infarction in connection with physical exertion and the risk has been estimated to be only 2.5 times greater than at rest for men who exercise regularly (>6 MET at least 4 - 5 days/week) (Mittleman *et al.,* 1993). For women, the risk of suffering a heart attack during and in connection with physical exertion is very small (compared with the risk during a randomly selected hour without physical exertion) and the small risk that has been reported appears to vanish with regular exercise. For both men and women who exercise regularly, the risk of having a heart attack at all (that is at any hour of the day) is less than half of that among untrained individuals (Alevizos *et al*., 2005).

It is known that intense physical exercise in children and adolescents, meaning mechanical loading on the skeleton, results in larger, stronger and more mineral dense bones and that this effect is more pronounced if the exercise is begun early (Kannus *et al*., 1995). If the exercise starts at an adult age, only small improvements in bone density are achieved. Nevertheless, it has clearly been shown that the risk of a hip fracture is lower among trained individuals, while evidence is accumulating (albeit less strong) that exercise at an adult age reduces other types of fractures related to osteoporosis (Karlsson, 2002). Interestingly, veteran cyclists with many years of training had significantly lower bone density than control persons of the same age and, although very physically fit, they had a higher risk of being affected by brittle bones with increasing age (Nichols *et al*., 2003). Among women, intense exercise training such as long distance running can also lead to diminished bone density (Balasch, 2003). There is also evidence that among adolescents, increased leisure time physical activity (i.e. outside structured school programmes) is significantly associated with fewer depressive symptoms (over a two year period) and accelerates learning by increasing cognitive processes (e.g. memory functioning) (Penedo *et al.,* 2005). In summary, the relationship between physical activity, health related fitness and health can be examined and understood by a model (Figure 1.4) by Bouchard & Shephard (1994) demonstrating the reciprocity between physical activity, fitness and health.

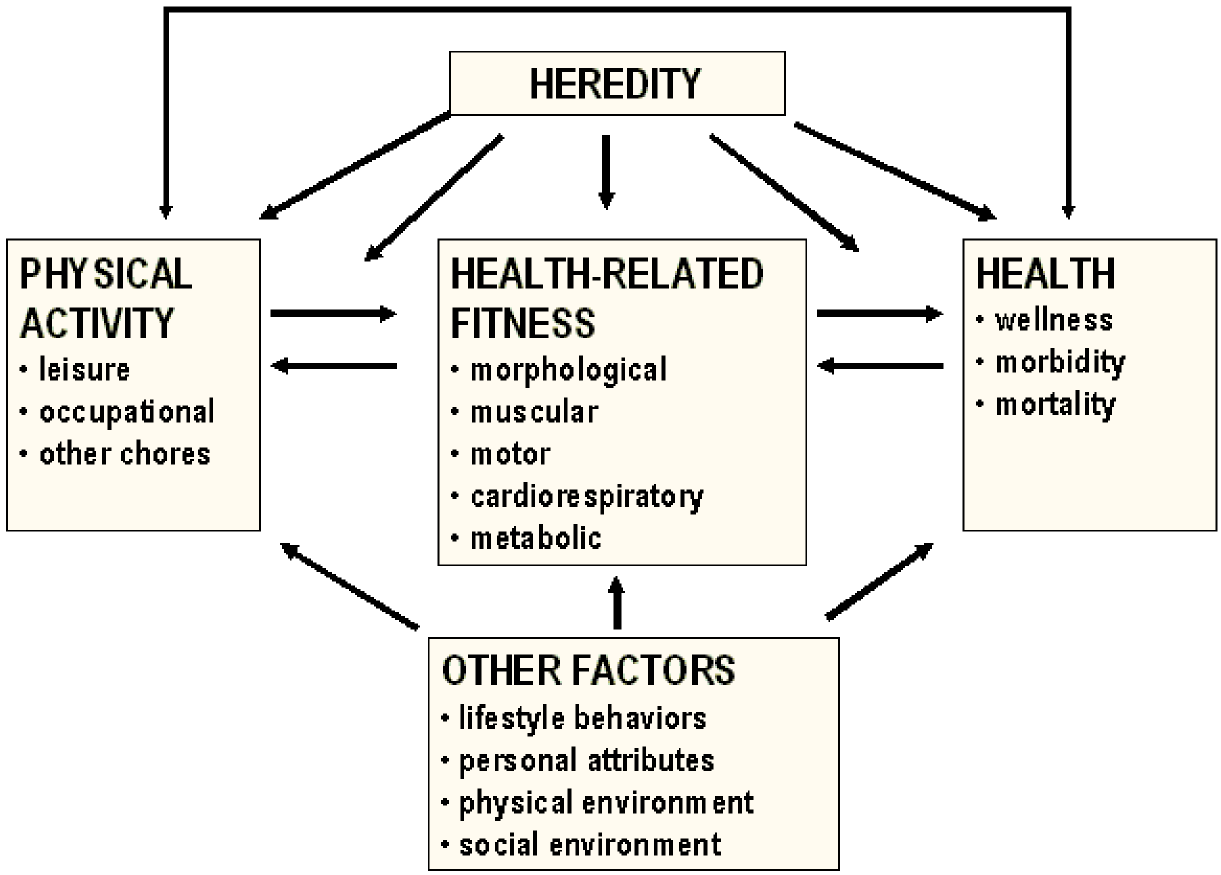


Figure 1.4: The relation between physical activity, fitness and health - Physical activity may influence fitness, which in turn may modify the level of physical activity. With increasing fitness, people tend to become more active, and the fittest persons tend to be the most active. The association between fitness and health is also reciprocal. Fitness influences health, but health status also influences both physical activity and fitness.

1.7: Physical activity guidelines:

It is becoming increasingly clear that variations in physical activity and sedentary behaviour are of enormous importance to the current and future health of children and adolescents (Strong *et al*., 2005). This is especially the case since it has been observed that several health outcomes related to physical activity, such as obesity tend to track from childhood into adulthood (Twisk *et al*., 1997) and therefore adequate participation in physical activity during childhood and adolescence may be critical in the primary prevention of chronic disease (Trost *et al*., 2006). Current interventions are therefore aimed at modifying physical activity and sedentary behaviour which in turn would favourably modify the risk profiles of chronic diseases such as type 2 diabetes, hypertension and cardiovascular disease (CMO, 2011). There is compelling evidence that an active and fit way of life has many important health benefits and that sedentary habits are associated with an increased risk of numerous chronic diseases and decreased longevity as shown in Figure 1.5 (DOH, 2004, Warburton *et al.,* 2010, CMO, 2011). In addition, strong and consistent evidence based on experimental studies for several health outcomes indicates that participating in 2 - 3 h of moderate to vigorously intense physical activity/week is associated with significant health benefits (Janssen & LeBlanc, 2010).

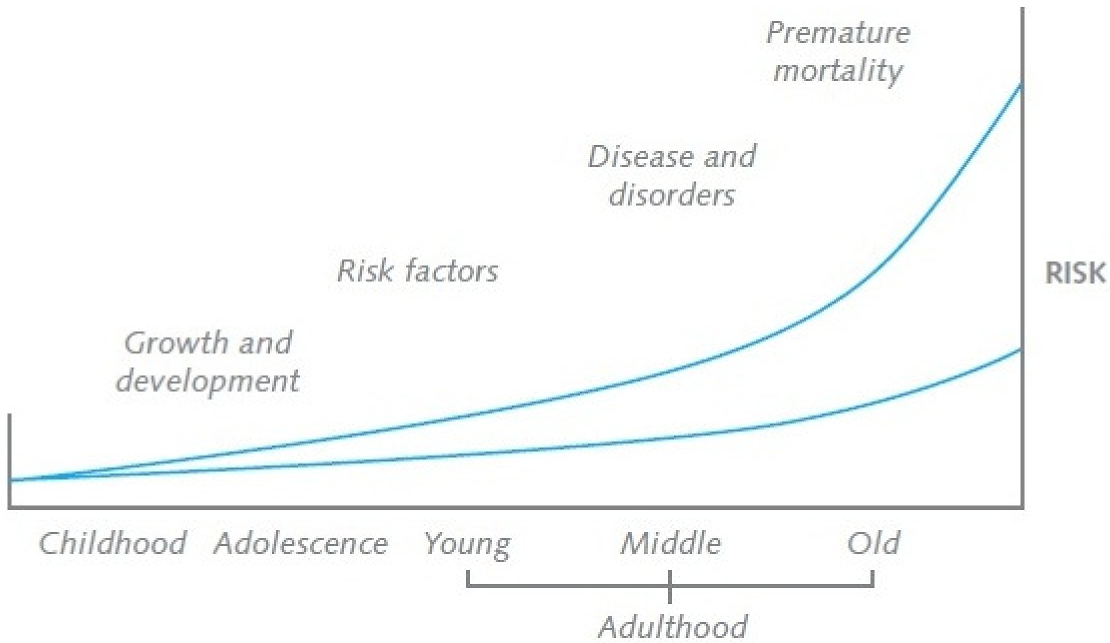


Figure 1.5: A hypothetical model of the key stages of disease development throughout the life course. The upper line on the graph represents theoretical rates of progression – through growth and development, development of risk factors, onset of disease and disorders, and premature mortality – for inactive individuals. The lower line represents active individuals. In this model, physical inactivity has negative effects at all stages of life in terms of impaired growth and development, or high risk factors, with the final expression as disease or early death being seen primarily from mid adulthood (DOH, 2004).

Evidence from observational studies also demonstrate dose response relations between physical activity and health (Janssen & LeBlanc, 2010), with differences in health risk between the least active (or fit) and inactive groups (Figure 1.5). However, continued debate as to how much, what type, how often, what intensity and how long the physical activity dose should be and how this dose should be quantified and disseminated has led to promulgation of numerous public health and clinical recommendations (Blair *et al.,* 2004). Some of the inconsistencies among physical activity recommendations are due to incomplete understanding of the principal mechanisms of physical activity in enhancing health, augmented by methodological differences in collecting and interpreting data, while some are due to a focus on different health outcomes by different groups (Blair *et al*., 2004). It is clear that moderate intensity exercise of at least 30 min/d as described in the consensus public health recommendations (USDHHS, 1996) produces significant improvements in health and that exercising at higher intensities has only modest additional effects (Blair *et al*., 2004).

The principal recommendation that persons accumulate ≥30 min of moderate intensity physical activity/day was largely directed at 40 - 50 million US adults who are sedentary and who account for much of the health burden of chronic disease (USDHHS, 1996). Since these persons are unlikely to have the physical capacity to engage in greater quantities of high intensity physical activity and because compelling evidence shows health benefits can be accrued with even moderate amount of regular exercise (Blair *et al*., 2004). The Centres for Disease Control (CDC)/American College of Sports Medicine (ACSM) consensus report recommended a dose of physical activity likely to be achievable by the primary target population and that was supported by a large evidence base as being efficacious for disease risk reduction among most persons (Blair *et al*., 2004). For example in the Harvard Alumni study, that examined physical activity in 16, 936 Harvard alumni showed an inverse relation between all cause mortality and increment of reported physical activity. The risk of death in men with an activity index of 3,500 kcal/week was less than 50% of that associated with <500 kcal/week. Furthermore, the men in the activity index of <2,000 kcal/week had 38% greater risk of death over a 12 y to 16 y follow up period than those in the higher activity index ranges (Paffenbarger *et al.,* 1986). The CDC/ACSM report also stated that persons meeting the basic recommendations could gain additional benefits by doing more exercise including some at higher intensities. Implicit in the ACSM recommendation is that exercise is similar to other therapeutic agents with a dose response characteristics of which a minimal dose that has proven efficacy and safety is typically prescribed as the initial dose (Blair *et al*., 2004). Recent evidence reaffirms the dose response relationship between physical activity and all cause mortality, since there is typically a risk reduction of around 30% for those achieving the recommended levels of at least moderate intensity physical activity on most days of the week, compared with those who are inactive (Lee *et al.,* 2001).

A sedentary lifestyle, often adopted during adolescence and continued into adulthood, is a major concern for public health (AAP, 2007, USDHHS, 2008, USDHHS, 2010). The dramatic increase in the prevalence of overweight and obesity and other lifestyle disorders such as diabetes, cancer, hypertension and cardiovascular diseases over the past decades (Trembly *et al*., 2005, Warbuton *et al.,* 2010) is related to, and often ascribed to lower levels of physical activity and increase in sedentary behaviour (Jakicic & Otto 2005, Ortega *et al*., 2007, WHO, 2010). This has resulted in the development of physical activity guidelines to promote maintenance of appropriate levels of physical activity and sedentary behaviour. Experts advocate promotion of physical activity among children and adolescents for health enhancement and to instil lifelong behavioural patterns that will result in more active and fit adult populations in the future (Sallis & Patrick, 1994, Twisk *et al*., 1997). This rationale rests considerably on two fundamental assumptions: first, that there are inherent acute physical and psychological benefits to physical activity among children and adolescents and, second, that physical activity behaviours between childhood and adulthood are correlated and that physically active children are more likely to grow up to be physically active adults compared with their inactive peers (Sallis & Patrick, 1994). These active adults will then be healthier by way of a reduced risk to a variety of health conditions (Sallis & Patrick, 1994). Although the evidence for tracking of physical activity behaviour is tenuous (Malina, 1996), most efforts for physical activity promotion among children and adolescents rely on the assumptions of tracking of physical activity from childhood to adulthood (Kohl *et al.*, 2000). The United Kingdom Expert Consensus Conference proposed that each child accumulate at least 60 min of at least moderate intensity physical activity each day (Cavill *et al.*, 2001, CMO, 2011) based on the findings that in general, differences in health outcomes have been observed at around 60 min/day in children and young people, whereas such differences have been observed at a level of approximately 30 min/d in adults. Thus, the best available evidence has been used in determining the level of activity required to benefit health in each specific age group (USDHHS, 2008, CMO, 2011).

The 2008 physical activity guidelines for Americans (USDHHS, 2008) recommend that children and adolescents should accumulate at least 60 min or more of physical activity daily. Most of the 60 or more min a day should be either moderate or vigorous intensity aerobic physical activity and should include vigorous intensity physical activity at least 3 days a week. As part of their 60 or more min of daily physical activity, children and adolescents should include muscle strengthening physical activity on at least 3 days of the week. In addition, children and adolescents should include bone strengthening physical activity on at least 3 days of the week. Similarly, the American Academy of Pediatrics (AAP) has published guidelines for enhancement of physical activity and limitation of sedentary behaviour in paediatric populations (AAP, 2007). It is recommended that television and video time is limited to a maximum of 2 h/d for the prevention of paediatric overweight and obesity and resultant comorbidities (AAP, 2007).

In the UK, recent published physical activity guidelines recommend that physical activity should be encouraged from birth, particularly through floor based play and water based activities in safe environments. Children of preschool age who are capable of walking unaided should be physically active daily for at least 180 min (3 h), spread throughout the day. All under 5s should minimise the amount of time spent being sedentary (being restrained or sitting) for extended periods (except time spent sleeping). In addition, the 180 min of recommended activity can be of any intensity. This aligns with the types of physical activity most naturally occurring during the early years, including intermittent and sporadic patterns. Moreover, the recommended 180 min of physical activity for preschool children who can walk can include light intensity activity, active play and more energetic activities, such as running, swimming and skipping. Furthermore, the 180 min of physical activity should be spread throughout the day rather than in one long session. For this age group, the amount of physical activity is more important than the intensity. In addition, this report recommends reduction of sedentary behaviour such as, time spent in infant carriers, car seats, highchairs and TV viewing or other screens based entertainment (CMO, 2011). Distinct guidelines were also published for children aged 5 - 18 y. It is recommended that this age group should engage in moderate to vigorous intensity physical activity for at least 60 min and up to several h/d, which is similar to the earlier guidelines (Cavill *et al.,* 2001). However, the current guidelines also recommend that vigorous intensity activities, including those that strengthen muscle and bone, should be incorporated at least three days a week. Additionally, all children and young people should minimise the amount of time spent being sedentary (sitting) for extended periods. Therefore, the current UK and US physical activity guidelines are consistent in recommending at least 60 min of MVPA daily and limitation of sedentary time for health promotion in children and adolescents.

1.8: Physical activity and the environment:

From an evolutionary perspective, humans are designed for a physically active lifestyle, while cultural circumstances permit and reinforce an inactive alternative in industrialised countries (Malina & Little, 2008). Throughout human evolution history, the lifestyle of humans included physical activity on a regular basis except for the past two or three generations (Malina & Little, 2008). Consequently, the combined effects of the transition to a sedentary lifestyle and attendant dietary changes have resulted first in an epidemic of coronary heart disease and more recently an epidemic of overweight/obesity in postindustrial societies (Malina & Little, 2008). Social and environmental changes have accompanied the ongoing rapid urbanisation in a number of countries during recent decades and therefore, understanding the role of urbanisation in the health risk transition is important for health policy development at national and local levels (Lim *et al*., 2009). Urbanisation is recognized as a driver of the globally changing health hazard panorama with specific proximate social, economic, environmental and behavioural health risks developing in the wake of urbanisation (Ompad *et al*., 2007). Low participation in health enhancing physical activity may be ascribed to urbanisation, which inevitably affects population health substantially (Hodge *et al*., 1995). Urbanisation is a global trend which may be altering habitual physical activity and sedentary behaviour of children and adolescents unfavourably but there is almost no objectively measured data on physical activity of urban and rural adolescents in Sub Saharan Africa. Therefore, one of the objectives of this thesis was to determine the impact of urbanisation (i.e. the environment) on physical activity levels, patterns and indices of adiposity in Kenyan adolescents.

In a study by Riddoch *et al* (2007) that examined the physical activity profiles of European children and adolescents, it was reported that only 2.5% of the 5,595 school children surveyed appear to have met recognized physical activity guidelines for children and adolescents. These disturbing findings reflect an ongoing decline in physical activity across all age groups during the past several decades in Europe (WHO, 2006) based on questionnaire based studies. This decline in physical activity may be explained by the mechanisation of work and daily tasks and thus, not labour intensive, the increased use of motorised transport instead of walking or cycling and increased sedentary behaviour such as inactive leisure pursuits (such as watching television and using a computer) (WHO, 2006). These trends are beginning to be replicated in the developing world. For example, it is estimated that by 2020 chronic diseases of lifestyle will be almost 50% of the burden of disease in Sub Saharan Africa (Kagwiza *et al*., 2005). Rapid urbanisation with changes in lifestyle, such as physical activity patterns could explain at least partially, the ongoing epidemiological transition in Sub Saharan Africa (Kagwiza *et al*., 2005).

1.9: Summary:

The literature review in this chapter has summarised the relationship between physical activity, energy expenditure and health. In addition, various approaches aimed at assessing physical activity have been described. It is apparent that objective methods are increasing the validity, reliability and accuracy of physical activity assessment such that the association between physical activity and health outcomes may be improved. Accelerometry is increasing in popularity as a method of choice in assessing physical activity and sedentary behaviour. However, there are several methodological challenges in using accelerometry such as which epoch and cutpoint to use, how much monitoring is required to obtain reliable estimates of physical activity in children and adolescents and the predictive validity of accelerometers need to be determined. In addition, the relationship between physical activity, energy expenditure and adiposity are explored in inactive and active cohorts. Finally, the impact of the environment and gender on physical activity and adiposity is also examined in this thesis.

1.9.1 Objectives:

1. To determine a) the effect of epoch and cutpoint selection on accelerometer counts per minute (CPM), sedentary and MVPA time, b) the minimum wear time required to achieve reliable estimates of CPM, sedentary and MVPA time in children and adolescents (Chapter 3).
2. To determine the validity of uniaxial ActiTrainer outputs and triaxial GT3X outputs against $\dot{V}O_2$ during structured activities in adolescents (Chapter 4).
3. To examine the validity of uniaxial ActiTrainer with HR monitoring vs. triaxial 3DNX accelerometers against DLW criterion method during free living activities in children and adolescents (Chapter 5).
4. To examine the relationship between free living physical activity, TEE, and indices of adiposity in children and adolescents (Chapter 6).
5. To examine the relationship between the environment, physical activity and indices of adiposity in children and adolescents (Chapter 7).

1.9.2: Thesis outline:

The first experimental chapter (Chapter 3) will examine the impact of methodological decisions on accelerometer outputs in European children (inactive) and Kenyan adolescents (active). The impact of several methodological decisions on processing of accelerometer data will be examined. Subsequently, the novel ActiTrainer, GT3X and 3DNX accelerometers will be compared against DLW and indirect calorimetry in children and adolescents (Chapter 4 - 5). The validated ActiTrainer will then be used in Chapter 6 to examine the relation between physical activity levels and patterns and free living energy expenditure and in Chapter 7, to determine the relation between the environment, physical activity and indices of adiposity in children and adolescents. Finally in Chapter 8, the findings will be put into perspective i.e., the prospects of accelerometry being the preferred method of choice in the objective assessment of physical activity and energy expenditure in children and adolescents.

Chapter 2

2.0: Materials and Methods:

2.1: Study design, recruitment and methods

This chapter is divided into three main parts. In the first part the overall study design is described in detail and the field work stage (including methods of recruitment and sample collection) are discussed. The second part describes the analytical processes involved in assessing energy expenditure using DLW technique. Finally, the third part describes physical activity assessment using accelerometers and indirect calorimetry.

2.1.1: The Identification and prevention of dietary and lifestyle induced health effects in children and infants (IDEFICS) study.

The IDEFICS study is one of the largest European studies of childhood obesity and includes 11 countries and approximately 17,000 children aged between 2 - 10 years (Bammann *et al*. 2006). The aim of the IDEFICS study is to investigate the primary factors leading to child obesity by assessing the lifestyle patterns of children within the European Union in order to develop suitable interventions aimed at countering the obesity epidemic. A number of novel interventions are utilised within the IDEFICS study in order to improve health awareness and encourage healthy eating and physical activity in children. One of the primary aims of IDEFICS is to validate field measures of physical activity (i.e. accelerometry) against criterion measures such as DLW in young children. This thesis adressess this objective. On the other hand, accelerometery may have limited utility in prediction of energy expenditure in relatively sedentary populations such as

European children in IDEFICS. Therefore, there is a need to validate accelerometers in active populations such as in Kenyan children (Larsen *et al*. 2004, Onywera *et al.* 2006).

2.1.2: The Kenyan adolescent physical activity study (KAPAS):

Kenyan children provide a model of very active childhood, with some authors reporting that Kenyan children from rural areas run up to 20 km to school and are actively engaged in leisure time activities and household chores (Larsen *et al*., 2004, Onywera *et al.,* 2006). Ideally, children (IDEFICS) and adolescent (KAPAS) study participants ages should have been matched. However, selection of older Kenyan adolescents was made based on practical reasons. Accelerometry is a novel technique (even more so in Africa). Older youths compared to very young children were therefore selected simply because of compliance issues and concerns over loss of devices. Compliance to study protocol becomes a major problem in studies of this nature where continuous monitoring for extensive periods is required, especially in very young children. This however, was not a problem in IDEFICS. The age differences between the two study cohorts was a minor limitation since the primary focus of this thesis was to validate accelerometers in two cohorts with distinct physical activity profiles.

2.1.3: Subjects

The fieldwork for the IDEFICS and KAPAS was carried out from October 2008 to July 2009 in convenience samples of healthy children (IDEFICS) and adolescents (KAPAS). 96 children aged 4 - 10 years were recruited from 4 validation centres at the universities of Glasgow (UGLW), Ghent (UGENT), Gothenburg (UGOT) and Zaragoza (UZAZ) for IDEFICS (Figure 2.1). KAPAS, on the other hand, recruited 266 adolescents aged 12 - 17 years from the Nandi region in Eldoret, Kenya (Figure 2.1) where habitual physical activity levels are thought to be high (Larsen *et al.,* 2004, Onywera *et al.,* 2006). The decision for taking convenience samples was made because the burden of taking measurements for participating children and adolescents was deemed too high for a random sample. All the schools used in the study were day schools, to examine the influence of active commuting to school on overall physical activity levels.

IDEFICS Study Area

KAPAS study area

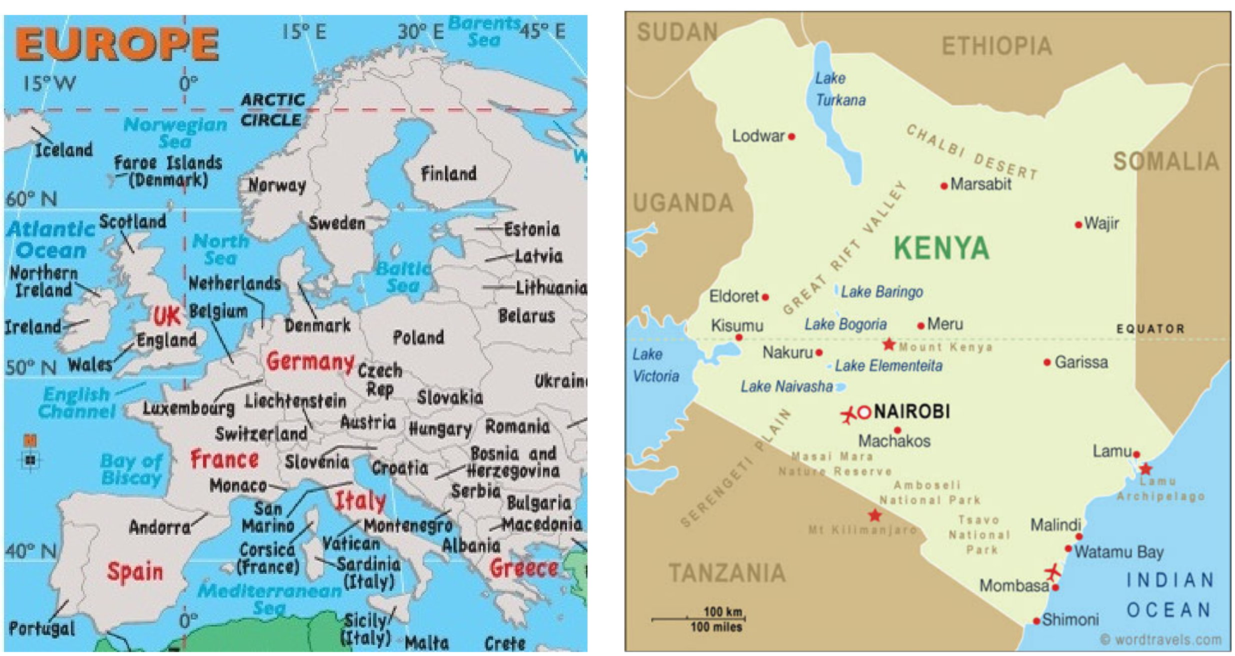


Figure 2.1: Geographic location of the IDEFICS (UK, Belgium, Sweden and Spain) and KAPAS (Eldoret, Kenya) study cohorts.

2.1.4: Sample size and power calculations:

For the IDEFICS sample size calculations showed that 31 children in each validation centre was sufficient for detecting mean differences of 5% between DLW criterion for AEE and accelerometer outcomes at a significance level of 0.05, with a statistical power of 80% for physical activity assessment methods (Bammann *et al*. 2011). However, data on which to base a sample size calculation for the KAPAS were limited as there have been no previous investigations on accelerometry and energy expenditure in Kenyan adolescents. Therefore, approximate sample size calculations to determine the required sample size to obtain statistical power of 80%, with a modest effective size of 0.33 indicated a minimum sample of 37. This was therefore set as the minimum recruitment target. The upper limit for participant numbers was determined by participant availability within the data collection time frame. Power calculations were completed using Minitab (version 14, Minitab Inc. Pennsylvania).

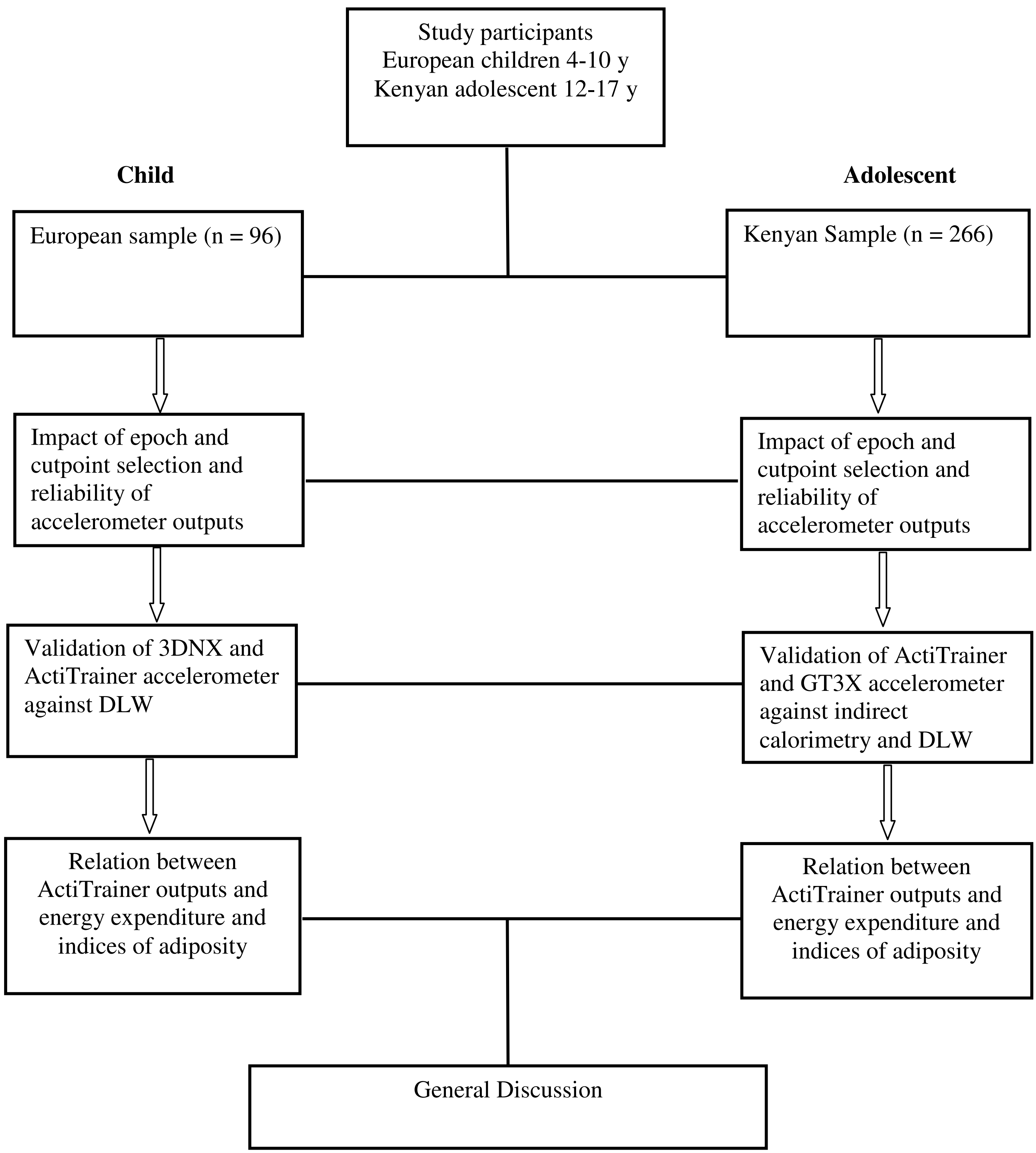


Figure 2.2: Flow diagram of thesis study design

2.1.5: Ethical approval:

Written informed consent was obtained from parents and the heads/principals of the participating schools. Ethical approval for the studies was granted by the respective Ethical Committees of each of the four centres for IDEFICS subsequently referred to as children. For KAPAS, subsequently referred to as adolescent, informed written consent was obtained from parents and the principals of the participating schools. Ethical approval for the studies was granted by the Institutional Research Ethics Committee (IREC), Moi University, Eldoret, Kenya. Subjects suffering from injury or any condition likely to limit physical activity were excluded from the studies. In addition, subjects were required to personally assent to participate in the studies. Furthermore, subjects were free to withdraw from the studies at any stage if they felt uncomfortable without the need to provide any explanation. However, none of the subjects withdrew.

2.1.6: Anthropometrics:

In all subjects, height was measured to the nearest 0.1 cm using a portable stadiometer (Somatometre Model SE V91, Seca, Birmingham, UK). The volunteer was measured barefoot, with their back positioned against a fixed backboard and their arms relaxed in the lateral position. The head was also positioned against the backboard, with the line of eyesight perpendicular to the backboard. Measurement was performed when the volunteer was positioned and relaxed, and a moveable headboard was lowered on to the top of the head with light pressure allowing hair compression. Body mass (BM) was also measured to the nearest 0.1 kg using portable weighing scales (Seca, Model 761, Vogel & Halke, Hamburg, Germany). Subjects were weighed with minimal clothing (e.g. shorts and shirt) without shoes and both feet flat on the balance, with arms positioned in the lateral position. BMI relative to International Obesity Task Force (IOTF) (Cole *et al*., 2000) definitions was used to define subjects as overweight or obese, and BMI z scores were calculated relative to WHO reference data (WHO, 2007a, WHO, 2007b).

2.1.7: Doubly labelled water:

TEE was measured with DLW according to the Maastricht protocol (Westerterp *et al.,* 1995) for the child cohort and the Aberdeen protocol (Speakman *et al.,* 1997) for the adolescent cohort. In short, the Maastricht protocols involves an overnight fast (about 8 h), collection of urine specimens before the administration of the isotope dose (day 0). This serves as the baseline isotope measurement. Subjects drink a weighed amount of $^{2}H_2{}^{18}O$ resulting in an initial excess body water enrichment of 150 ppm for deuterium and 300 ppm for oxygen 18. Subsequent urine samples are collected from the second voiding in the morning and a subsequent voiding in the evening on Days 1, 4, and 8. Samples were analysed in duplicate for H_2O^{18} and $^{2}H_2O$ at the Department of Human Biology at Maastricht University, Netherlands. The Aberdeen protocol on the other hand, involves collection of baseline urine samples before oral dosing with 0.15 g H_2O^{18} and 0.12 g $^{2}H_2O$/kg body mass. The following morning, subjects provide two timed urine samples (morning and evening). This is repeated over the 7 d urine collection period. Samples were analysed in duplicate for H_2O^{18} and $^{2}H_2O$ at the Energy Metabolism Research Unit in the Department of Nutritional Sciences at The University of Aberdeen, Scotland.

(i) Urine samples: All urine samples were collected in non acidified plastic bottles. The urine was aliquoted immediately into smaller plastic tubes (about 5 ml urine) and stored frozen (-4°C) until analysis.

(ii) Analytical Methods:

All isotope measurements were made with an isotope ratio mass spectrometer (OPTIMA, Micromass Inc., Beverly, USA). The deuterium measurement use zinc reduction of the water to hydrogen gas at 490^{o}C. For the zinc equilibration method, hydrogen gas was prepared from the samples using an offline zinc reduction method 3 µL of sample (water, diluted dose, or urine) were dispensed into quartz reduction vessels over a stream of nitrogen gas into the base of the vessels containing 100 mg of zinc. Samples were frozen with liquid nitrogen, and the vessels were evacuated and then sealed. Reduction of the samples was achieved by heating the vessels at 500°C for 30 min. The resulting hydrogen gas was introduced into a dual inlet, stable isotope ratio mass spectrometer (OPTIMA, Micromass Inc., Beverly, USA) and analyzed for deuterium enrichment. The ^{18}O measurements determine $C^{18}O_2$ using a H_2O-CO_2 equilibration system. 2 ml of urine was then equilibrated with 10 ml tank CO_2 in a sealed tube. The delta deuterium and oxygen 18 values for the predose (δ_{pre}) and postdose (δ_{pos}) were then determined. The DLW dose was subsequently diluted with tap water. The amount of dose diluted and water used was recorded.

The deuterium and oxygen 18 content of the tap water ($\delta_{tap)}$ and diluted dose ($\delta_{dose)}$ were measured.

$X = [(\delta_{post\ -}\ \delta_{pre})/\ \delta_{dose} - \delta_{tap}]\ X\ 18.02a/WA$

Where W = amount of water in g used to dilute the dose, A = amount of dose in g administered to the subject, a = amount of dose in g diluted for analysis. Linear regression was used to calculate the slope and intercept of the linear relationship between the time in days and the normalised data for each isotope. The pool sizes N_H and N_O were derived as the reciprocal of the intercept. The intercept of the regression line is the ratio of the pool sizes spaces $N_H/N_{O.}$ The multipoint data was plotted to inspect for any outliers. Any outliers were reanalyzed.

(iii) Calculation of daily CO_2 production: The mean daily CO_2 production (rCO_2) in mol/d was calculated according to the revised equation of Speakman *et al.* (1993).

$rCO_2 = (N/2.196) \times (k_O - 1.042\ k_H)$ where $N = [(N_O) + (N_H/\ 1.0427)]/2$.

(iv) Determination of Total energy expenditure:

The estimate of energy expenditure was calculated from carbon dioxide production assuming 127.5 kcal/mol carbon dioxide (a typical western diet will produce Respiratory Quotient (RQ) of 0.85, with 15% of energy from protein oxidation). The use of a general value for conversion of CO_2 to energy expenditure for a western type diet was found to predict to within 5% of energy expenditure in a random sample of individuals (Black *et al*. 1986).

The Weir Equation uses measured RQ and urinary nitrogen production rate (UN) to calculate energy expenditure (Q) was used as shown below.

$Q = 3.941\ (rCO_2/RQ) + 1.106\ RCo_2 - 2.17\ UN$

(v) Determination of Basal Metabolic Rate, Activity induced Energy Expenditure and Physical activity Levels:

The Schofield equations (1985) based on gender, age, height and weight were used to predict BMR for the child sample aged 3 - 10 years as described below.

BMR (kcal/d) = 19.59W + 1.303H + 414.9 (males)

BMR (kcal/day) **=** 16.969W + 1.618H + 371.2 (females)

W is weight in Kg and H is height in cm.

The predicted BMR above, were subsequently used to predict activity induced energy expenditure (AEE). AEE is the energy expenditure associated with muscular contractions involved in body movements and maintenance of posture, and is the most variable component of TEE and is expressed as = 0.9% x TEE – BMR and Physical activity Level (PAL) expressed as: = TEE/BMR (Westerterp, 2003) in the child sample only. Ethnicity may have an impact on BMR, as it has been reported that Africans have lower BMR values compared to Caucasians (Vander Weg *et al.,* 2004, Henry, 2005). Predictive equations developed for Caucasians may therefore lead to significant overestimation of BMR. Therefore, the predictive validity of the Schoefield equation in African populations is questionable (Wong et al., 1996, Vander Weg *et al.,* 2004, Henry 2005). Currently, there is no conclusive study on the predictive validity of any of the multiple published BMR prediction equations that would be appropriate to use in adolescents of Sub Saharan African descent. For the adolescent cohort therefore, PAL and AEE were not calculated from any of the published predictive equations as these were deemed to be inaccurate based on the fact that an appropriate BMR prediction equation for African adolescents is yet to be conclusively determined. Therefore, only measured TEE was reported in this thesis.

2.1.8: Accelerometry:

The ActiTrainer accelerometer (ActiGraph LLC, Pensacola, FL, USA) is surrounded in a metal shield and packaged into a plastic enclosure measuring 5 x 4 x 1.5 cm, weighs approximately 45 g including a 3V (2430) coin cell lithium battery, has a sampling range of 0.25 to 2.5 *g*, a sampling frequency of 30 Hz and contains a cantilevered rectangular pizoelectric bimorph plate and seismic mass, a charge amplifier, analog bandpass filters, and a voltage regulator to measure acceleration in a single plane (Figure 2.3). The filtered acceleration signals (in the vertical plane) generate counts that are digitalised and the magnitude is summed over a user specific time (an epoch interval). At the end of each epoch the summed value is stored in memory and the numerical integrator reset.

Figure 2.3: Uniaxial ActiTrainer ActiGraph accelerometer

The GT3X accelerometer (ActiGraph LLC, Pensacola, FL, USA) on the other hand, is a triaxial accelerometer which may allow for a more comprehensive analysis of activity and movement patterns. The GT3X has the same dimensions as the GT1M (ActiGraph LLC, Pensacola, FL, USA) (27g, 1.5 x 1.44 x 0.70 cm and records accelerations ranging from 0.5 - 2.5 *g*'s. Its output is digitized at a rate of 30 Hz and the magnitude is summed over a user specific epoch (Figure 2.4). The data outputs from GT3X are the sum of the three axes = (X +Y+Z), where Y are counts in the vertical plane, X are counts in the anteroposterior plane and Z are counts in the mediolateral plane.

Figure 2.4: Triaxial GT3X ActiGraph accelerometer

The Triaxial 3DNX^{TM} model v3 accelerometer (BioTel Limited, Bristol, UK: www.biotel.co.uk) on the other hand, is also sensitive to movements in three planes: X (anteroposterior), Y (mediolateral) and Z (vertical). The unit measures 54 x 54 x 18 mm, and weighs 70 g including a 3.6 v lithium battery (Saft Ltd., UK) with a life of ~21 days collecting data at 5 s epoch (Figure 2.5). The unit contains two ADXL321 biaxial microelectromechanical (MEMS) sensors (Analog Devices Ltd., Surrey, UK) positioned orthogonally to measure acceleration in three movement planes. The sample frequency of the 3DNX is 100 Hz with a low pass filter set at 0.2 Hz and a high pass filter set at 20 Hz which ensures that most non human movement, such as vibration, is not registered. Once the digital signal is filtered, it is fully rectified and integrated over one second. The 1 s integrals are summed depending on the epoch selected by the user (currently between 5 and 60 s) to produce an arbitrary ‘counts’ value. Approximately 42 days of data can be stored when collecting at 5 s intervals. All raw accelerometer data were downloaded using dedicated software (Biotel Limited, Bristol, UK) and imported into a spreadsheet where the 15 s epoch accelerometer counts from X, Y and Z axes were summed.

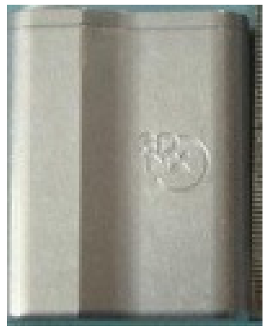


Figure 2.5: Triaxial 3DNX Biotel accelerometer.

Prior to testing of each subject, activity monitors were calibrated by the manufacturers, tested and fully charged. The accelerometers were placed in a small nylon pouch and firmly adjusted at the right hip of the subject by an elastic belt. Activity diaries were also completed by each subject or their parent(s)

Figure 2.6: Child and adolescent subjects wearing accelerometers and HR monitors.

(i) Accelerometer data editing and analysis: Accelerometer data were analysed using algorithms developed in R (version R 2.9.0. R Foundation for Statistical Computing, Vienna, Austria: http://www.R-project.org). A very large number of statistical analyses are implemented as add on packages to R, most of them are freely available from public repositories (Comprehensive R Archive Network = CRAN, http://cran.r-project.org/, Bioconductor www.bioconductor.org) and very easily installable from within R. Implementing the software for accelerometer data analysis within a widely used statistical environment such as R has an important advantage over the already existing computing infrastructure (e.g., for handling dates and times, computing summary statistics, and making custom high quality plots). The advanced data analysis capabilities of R can easily automate any complex analyses. These functions allows R: to read in raw data files (currently different versions of ActiGraph and 3DNX raw files but new file types can be easily added as required), apply simple data cleaning algorithms (e.g., filter out implausible data, find non wear periods such as 20 or any other number of minutes of consecutive zeros), calculate summary statistics (wear time, counts per minute, time in activity intensity zones using unlimited number of different cutpoints, time in MVPA bouts) by any period (usually day or hour but potentially also periods like 30 min or 2 days) and make corresponding diagrams in any graphics format supported by R (e.g., pdf, eps, jpeg, png, etc). R also allows customisation of automatic tasks, such as producing feedback to the participants in the form of tables, figures and texts. For the child and adolescent studies, a set of add on functions to R were developed which allowed R to automatically read in the accelerometer raw files, reintegrate data collected in 1 s epoch to 15 s, 30 s and 60 s, edit the data for excluding the likely non wearing periods and compute daily summary statistics. Two rules were used for excluding data: (a) all negative counts were replaced by missing data code and (b) periods of 20 min or more consecutive zero counts were replaced by missing data code prior to further analysis as recommended by Treuth *et al*. (2003) who found this period of inactivity to be inconsistent with monitor wear. The output generated by R included, CPM, HR data, total monitoring time, number of min spent in each physical activity intensity for each cutpoint and percentage of overall time spent in the specified activity level.

2.1.9: Heart rate assessment:

All subjects in the child and adolescent studies were fitted with a HR transmitter belt (Suunto t6, Suunto Oy, Vantaa, Finland) on the chest to record HR continuously on the ActiTrainer accelerometer. HR data can be added to the list of ActiTrainer accelerometer output parameters to record average, peak and instantaneous beats/min. Before wearing the HR monitor, the electrodes were moistened with running water. The chest strap was securely attached to the chest to create a unique coded communication link with the ActiTrainer accelerometer. To ensure trouble free HR monitoring, subjects were instructed not to wear HR monitors where electromagnetic disturbances (computers, mobile phones and cars) would interfere with signal transmission. An explorative approach was used to test the relationship between energy expenditure and HR indices. The HR indices tested were mean HR, median HR, flex HR (defined as the average of the lowest HR during exercise and the highest HR during rest (Trost, 2001). In addition, accelerometer data was used to define HR in all the activity domains (sedentary, light, moderate and vigorous) using the Evenson cutpoints (2008). In addition, the difference in HR during vigorous and sedentary activity, moderate and sedentary activity and light and sedentary activity was examined. None of the HR parameters examined were significantly associated with energy expenditure except the difference in HR during moderate and sedentary activity which was defined as (ΔHR_{acc}), hence ΔHR_{acc} was the only HR parameter subsequently used in energy expenditure prediction equations in this thesis.

2.2.0 Global positioning system (GPS):

Subjects were fitted with either a Timex trainer V 1.3.36 (Timex Group, USA) or GPSports Team AMS Release 1.2.1.12 (Fyshwick, Australia) GPS devices during their travel from home to school to obtain daily distance travelled to school (active commuting) in the adolescent sample only. The position of a GPS receiver is calculated by measuring the distance between itself and three or more GPS satellites. Each satellite is equipped with an atomic clock. When first powered on, GPS devices undergo an initialization period, during which they acquire signals from the satellites, and synchronize the GPS clock with the satellite's atomic clock. GPS devices constantly receive and analyze radio signals from the satellites, calculating precise distance (range) to each satellite being tracked by using trilateration, a mathematical technique used to determine user position, speed, and elevation (Maddison & Mhurchu, 2009).

2.2.1: Metabolic gas analysis:

Expired respiratory gases were collected and $\dot{V}O_2$ measured by an online breath by breath system using a portable gas analyzer (Cosmed K4b^2, Rome, Italy) in the adolescent sample only. This portable indirect calorimetry system was worn on the back using a harness. The unit is light weight (approximately 1.5 kg) and is specifically designed to measure $\dot{V}O_2$ in non laboratory settings (Trost *et al.,* 2006). A flexible facemask (Hans Rudolf, Kansas City, MO, USA) held in place by a nylon head harness covered the participant's nose and mouth. The mask was attached to a bidirectional flow meter to measure the volume of inspired and expired gases. A sample line running from the turbine to the analyzer unit delivered expired air for the determination of O_2 concentration (Figure 2.7). The Cosmed K4b^2 has been shown to provide valid measures of $\dot{V}O_2$ over a range of exercise intensities in children and adults (McLaughlin *et al.,* 2001, Eisenmann *et al.,* 2004, Trost *et al.,* 2006). The Cosmed K4b^2 was calibrated immediately before each test according to the manufacturer's guidelines. The guidelines consisted of a four step calibration process: ambient air calibration, reference gas calibration, delay calibration, and turbine calibration. The ambient air calibration was automatically run before tests to correct for atmospheric values. Subsequently, a reference gas calibration was then done using reference gases of known composition (5.5% CO_2, 15.9% O_2, and 78.6% N). A delay calibration was then performed to compensate for the time lag between the expiratory flow measurement and the gas analyzers. The final calibration step, the turbine calibration, included setting the flow meter with a 3 L syringe (Hans Rudolph, Kansas City, MO, USA) to ensure accurate volume measurements.

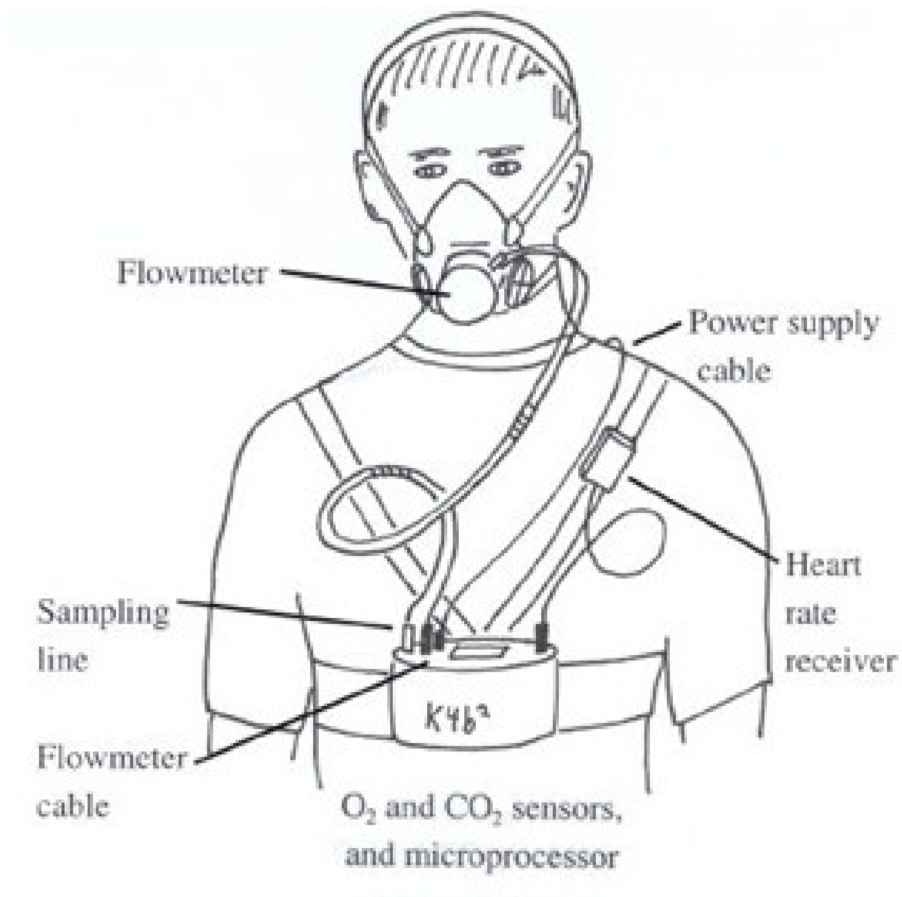


Figure 2.7: Subject wearing Cosmed K4b^2 gas analyser.

2.3: Statistical analysis:

Data were analyzed using SPSS (version 17), R (version R 2.9.0., R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org) and Minitab v. 15. Prior to analysis, quantitative data were tested for normality using the Shapiro Wilk normality test and appropriate statistics employed. Specific statistical analyses used are described in detail in the method section of each chapter.

Chapter 3:

3.0: Impact of methodological decisions on accelerometer outcome variables in children and adolescents.

3.1 Introduction:

Objective assessment of physical activity in children and adolescents by accelerometry is considered valid, precise and reliable when assessed in a number of different populations (Rowlands & Eston, 2007, Trost, 2007, Stone *et al*., 2009). However, there is considerable inconsistency in accelerometry methodology as different studies use different epoch and cutpoints for physical activity classification (Guinhoya *et al*., 2006, Reilly *et al*., 2008). Furthermore, accelerometer data may not be measured in uniform periods each day (Corder *et al*., 2008) and therefore, it is imperative that accelerometer studies determine what constitutes reliable estimates of habitual physical activity and sedentary time.

A significant impediment to the universal application of accelerometer derived outcome variables in comparative studies is the lack of concordance in the preanalytical and analytical processing protocols employed in different accelerometer studies (Esliger *et al*., 2005, Colley *et al*., 2010). It has been reported that small methodological inconsistencies such as use of different epochs and cutpoints can have a profound influence on accelerometer outcome variables (Rowlands *et al*., 2006, Reilly *et al*., 2008). Furthermore, different authors use different decision rules to define, how many hours constitutes a valid days' recording, how many consecutive zeroes define a period when monitor was not worn and how many days of monitoring are required to achieve reliable estimates of habitual physical activity levels (Màsse *et al*., 2005, Ward *et al*. 2005). Consequently, it has been recommended that protocols for use in paediatric and adolescents accelerometer studies should be standardised to eliminate this potential source of variability in accelerometer studies (Trost *et al*., 2005, Ward *et al*. 2005, Reilly *et al*. 2008) and thus assess physical activity and sedentary behaviour levels accurately and reliably between and within populations.

The most appropriate choice of accelerometer epoch and cutpoint for physical activity assessment in children and adolescents is currently equivocal (Stone *et al*., 2009) as relatively few studies have addressed this and only within a narrow range in terms of age and settings (Nilsson *et al*., 2002, Penpraze *et al*., 2006b, Rowlands *et al*., 2006, McClain *et al*., 2008, Dorsey *et al*., 2009, Edwardson and Gorely, 2010). Recent evidence suggests that choice of epoch and cutpoints may interact and influence physical activity classification in an unexpected manner. For example, McClain *et al* (2008) reported increased MVPA time with 60 s epoch compared to 5 s epoch, but attributed this finding to the brief monitoring time of physical activity and the use of the Freedson cutpoints. Edwardson and Gorely (2010) observed the opposite trend, that is, decrease in observed MVPA time with shortening of epoch duration. Furthermore, Reilly *et al* (2008) reanalysed data using different epochs and cutpoints for sedentary time and MVPA and found values ranging from 180 - 501 min of sedentary time per day and 28 - 266 min of MVPA per day for the same periods and individuals. Thus some authors report a pronounced effect of epoch on physical activity and sedentary time (Reilly *et al*., 2008), where as other authors report a modest epoch effect (Dorsey *et al*., 2009).

Several studies have examined the number of days of physical activity monitoring needed to obtain a reliable estimate of habitual physical activity (Trost *et al*., 2000, Penpraze *et al*., 2006, Baranowski *et al.,* 2008). The objective of these studies has been to identify the minimum number of days needed to measure physical activity to minimize participant burden. This is usually done using the intraclass correlation coefficient (ICC) with values ranging from 0.7 - 0.9 being considered acceptable reliability. However, the results of these studies are highly dependent on the variability of the data. Recent studies have reported a wide range of criteria for selecting a valid day (i.e., an accurate estimate of a single day's physical activity) from as few as 6 h/d (Penpraze *et al*., 2006) and up to 16 h/d (Slootmaker *et al*., 2009). On the other hand, there are a variety of criteria for selecting the number of h/d one should wear an accelerometer to reflect daily free living physical activity (Catellier *et al*., 2005, Troiano *et al*., 2007, Matthews *et al*., 2008, Slootmaker *et al*., 2009, Matthews et al., 2012). Slootmaker *et al*. (2009) used an assumption that people sleep 8 h/d and therefore restricted valid days to persons with more than 16 h of monitoring. This clearly would not apply to young children such as in IDEFICS since they may sleep for more than 8 h in a day and their sleeping patterns may be intermittent. Another approach that has been used to determine accelerometer wear time is to normalize each person's total minutes of daily activity to a 12 h/d to balance different amounts of wear time (Young, *et al*., 2009). However, this approach may create an over or underestimation of actual movement time. A common approach, including that used in U.S. National Health and Nutrition Examination Survey (NHANES) accelerometer analyses, is to require 10 or more h/d of accelerometer wear time to be considered a valid measurement day (Troiano *et al*., 2007, Matthews, *et al*., 2008). On the other hand, Catellier *et al.* (2005) recommended use of the 70/80 rule. This rule provides a sample specific recommendation based on 70% of the sample having accelerometer data. A valid day is then defined as 80% of that observed period.

It is therefore apparent that practical and analytical methodologies used in accelerometry studies are inconsistent and thus it is reasonable to expect that this would have a profound impact on accelerometer outcome variables to varying degrees. The aim of this study therefore was to examine how the classification of CPM, sedentary and MVPA time varies according to epoch length when assessed using different published physical activity intensity cutpoints. A secondary aim was to examine the reliability of accelerometer outcome variables (CPM, sedentary and MVPA time) over several hours and days of measurement in a cohort of young European children and Kenyan adolescents.

3.2 Methods

3.2.1 Subjects:

96 children (50 female, 46 male) aged between 4 - 10 years were recruited from 4 validation centres at the universities of Glasgow (UGLW), Ghent (UGENT), Gothenburg (UGOT) and Zaragoza (UZAZ) and 266 Kenyan adolescents (132 boys, 134 girls) aged between 12 - 17 y were also recruited (see section 2.1.3 for details).

3.2.2 Physical activity assessment:

Free living sedentary and physical activity times were objectively assessed using the ActiTrainer uniaxial accelerometer (ActiGraph, Pensacola, FL, USA). The monitor was set to record physical activity in a 1 s epoch. Following collection, data were reintegrated to 15 s, 30 s and 60 s epochs. Subjects wore the accelerometer for seven consecutive days between January and April 2009 during school term time. Accelerometers were mounted on the right hip of each subject by an elastic belt and adjusted to ensure close contact with the body. Parents were also asked to complete a daily activity or 'non wear' diary during the 7 day monitoring period with instructions to record the time the accelerometer belt was attached and removed. Subjects were required to wear the accelerometer from the moment they woke up in the morning until bed time in the evening so that a full day of physical activity and sedentary time could be assessed. Data were analysed using previously published cutpoints (Puyau *et al*., 2002, Reilly *et al*., 2003a, Sirard *et al*., 2005, Pate *et al*., 2006, Evenson *et al*., 2008). For sedentary activities these were: Sirard: <398 counts/15 s, Reilly: <1100 counts/min, Puyau <800 counts/min, Evenson: <100 count/min and for MVPA these were Sirard: >890 counts/15 s, Pate: >420 counts/15 s, Puyau: >3200 counts/min and Evenson: >2296 counts/min. Specific cutpoints were either divided down or multiplied up for data reintegrated to 15 s, 30 s and 60 s epochs to calculate time spent sedentary and in MVPA.

3.2.3 Reliability of accelerometer variables over several days of measurement:

Previous studies have recommended a minimum of at least 6 days including at least 1 weekend day of valid recording of at least 360 min of continuous monitoring per day (Trost *et al*., 2000, Penpraze *et al*., 2006a). The reliability of these inclusion criteria as well as various other inclusion criteria was assessed in this study. Reliability coefficients for accelerometer outcome variables (CPM, sedentary and MVPA time) over several days and hours of monitoring were computed using the ICC defined below.

(1) $ICC_s = \sigma_b^2 (\sigma_b^2 / (\sigma_b^2 + \sigma_w^2)$

Where σ_b^2 is the between subject variance component and σ_w^2 is the within subject variance component.

Reliability was also predicted using the Spearman Brown Prophecy formula (Trost *et al*., 2005) which uses ICC as a measure of reliability as defined below.

(2) $N = [ICC_t / (1 - ICC_t)] [1 – ICC_s/ICC_s]$

Where N is the number of measures or days needed, ICC_t is the desired level of reliability (typically 0.7 to 0.9) and ICC_s is the single day reliability (Trost *et al*., 2005, Baranowski *et al.,* 2008).

3.2.4 Data analysis:

Data were expressed as mean (standard deviation (SD)) or median [range] following a Shapiro Wilk test for normality. CPM and time classified as sedentary was normally distributed. Statistical analysis to determine differences in the classification of CPM and sedentary time across the different epochs was carried out using Generalised Linear Model approach (repeated measures ANOVA). Mauchly's test was used to test the assumption of sphericity and if violated degrees of freedom were corrected using Greenhouse Geisser estimates of sphericity if epsilon <0.75 or the Huynh Feldt correction if epsilon >0.75, followed by Bonferroni *post hoc* test. Significance was set at $P<0.05$. Time classified as MVPA was not normally distributed and therefore the Kruskal Wallis test followed by Mann Whitney U tests was used to test for group differences. Significance was set at $P<0.05$. All statistical analysis was completed using the software package SPSS, Version 17.0 (SPSS, inc., Chicago, IL).

3.3 Results:

Descriptive characteristics of the child and adolescent subjects are shown in Table 3.1 and Table 3.2

Table 3.1: Descriptive characteristics of child subjects (mean± SD).

	All	Females	Males
Centre			
UGENT	38	25	13
UGLW	19	7	12
UGOT	10	3	7
UZAZ	29	15	14
All	96	50	46
Height (m)	1.22 ± 0.1	1.19 ± 0.1	1.25 ± 0.1
Weight (kg)	25.8 ± 7.9	24.9 ± 7.2	26.8 ± 7.9
BMI (kg/m^2)	17.2 ± 3.9	17.3 ± 3.2	16.9 ± 4.0
Monitoring time (min)	697 ± 111	678 ± 81	712 ± 110

Table 3.2: Descriptive characteristics of adolescent subjects (mean±SD).

	All	Females	Males
Subjects	266	134	132
Height (m)	1.60 ± 0.1	1.61 ± 0.1	1.65 ± 0.1
Weight (kg)	44.0 ± 7.0	43 ± 6.3	42 ± 7.5
BMI (kg/m^2)	18.0 ± 3.0	16.2 ± 2.5	15.0 ± 2.0
Monitoring time (min)	685 ± 72	750 ± 177	620 ± 94

In children, subject adherence remained above ≥90% for the first 6 days of the week long study period (Table 3.3). The compliance fell to 52% on day 7 as, for practical reasons, accelerometers were collected from children at the UZAZ centre when children completed the time consuming final body composition and had to remove the accelerometer. If final day data from UZAZ are removed from the adherence analysis, compliance on the final day remained high at 75% (Table 3.3). In adolescents on the other hand, subject compliance systematically declined with increasing monitoring period. Compliance fell to only 12% for the 7 day monitoring protocol (Table 3.3).

Table 3.3: Subjects with ≥6 h of data during the 7 day monitoring period for the child and adolescent cohorts

	Children							Adolescents						
	1 d	2 d	3 d	4 d	5 d	6 d	7 d	1 d	2 d	3 d	4 d	5 d	6 d	7 d
Subjects	96	92	90	87	87	86	50	266	266	235	204	127	106	32
% Compliance	100	96	94	91	91	90	52	100	100	88	77	48	40	12

3.3.1 Reliability of accelerometer variables over several hours and days of measurement in children and adolescents:

Table 3.4 shows the reliability coefficients of accelerometer variables over several hours of monitoring in children and adolescents. Single day ICC for a minimum of 6 h of monitoring in children for CPM was 0.32, 0.33 for sedentary time and 0.35 for MVPA. Therefore, in children, the number of days (including at least 1 weekend day) required to obtain 80% reliability for average CPM, sedentary and MVPA was 8.5 days, 8.1 days and 7.4 days, respectively. On the other hand, single day ICC for a minimum of 12 h of monitoring for CPM was 0.17, 0.16 for sedentary time and 0.13 for MVPA. The number of days (including at least 1 weekend day) required to obtain 80% reliability for average CPM, sedentary and MVPA was 19.5 days, 21 days and 26.8 days, respectively (Table 3.5). In adolescents on the other hand, single day ICC for CPM for a minimum 6 h monitoring was 0.47, for sedentary time it was 0.48 and finally for MVPA it was 0.44 (Table 3.4). 80% reliability for CPM was calculated as 4.5 days, for sedentary time was 4.4 days and for MVPA it was calculated as 5 days (Table 3.5). On the other hand, single day ICC for a minimum 12 h monitoring for CPM was 0.37, for sedentary time was 0.38 and for MVPA was 0.38. The predicted number of days of monitoring to obtain 80% reliability was therefore calculated to be 6.5 - 6.8 days (Table 3.5). In both the child and adolescent cohorts a minimum of 6 h of monitoring was sufficient to reliably estimate habitual physical activity. Therefore, subjects without a minimum of 6 h monitoring were not included in the subsequent analysis in this thesis.

Table 3.4: Reliability of accelerometer outcome variables over several hours and days of measurement for child and adolescent cohorts

	ICC[a] Children				ICC[a] Adolescents			
	6 h	**8 h**	**10 h**	**12 h**	**6 h**	**8 h**	**10 h**	**12 h**
CPM	0.32	0.32	0.28	0.17	0.47	0.44	0.39	0.37
Sedentary	0.33	0.29	0.21	0.16	0.48	0.45	0.4	0.38
MVPA	0.35	0.29	0.24	0.13	0.44	0.45	0.4	0.38

ICC Intraclass correlation coefficient (intraindividual/total variation).
a Based on 6 days (defined here as ≥6 h, ≥8 h, ≥10 h and ≥12 h) of monitoring including at least 1 weekend day.

Table 3.5: Number of days required to obtain 80% reliability in child and adolescent cohorts

	Days of Measurement[b] Children				Days of Measurement[b] Adolescents			
	6 h	**8 h**	**10 h**	**12 h**	**6 h**	**8 h**	**10 h**	**12 h**
CPM	8.5	8.1	10.1	19.5	4.5	5.1	6.3	6.8
Sedentary	8.1	9.8	15	21	4.3	4.9	6	6.5
MVPA	7.4	9.8	12.7	26.8	5.1	4.9	6	6.5

b Predicted by Spearman Brown Prophecy formula.

3.3.2 Variation in accelerometer outcome variables across epochs for different cutpoints

In children, choice of epoch had no significant effect on average CPM (F (1, 582) = 0.05, P=0.78) for 15 s, 30 s and 60 s epoch (577 ± 193, 576 ± 193 and 578 ± 196, respectively. Similarly, for adolescents, choice of epoch had no significant effect on average CPM F (11, 2846) = 1.57, P = 0.79) for 15 s, 30 s and 60 s epoch (785 ± 226, 788 ± 234 and 791 ± 241, respectively.

Sedentary time: Choice of epoch and cutpoints had a significant effect on sedentary time (F (1.3, 763) = 254.7, P<0.05) for children. *Post hoc* analysis revealed significantly (P<0.05) less sedentary time per day using Reilly cutpoints when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs: 570 ± 91 min vs. 579 ± 93 min and 570 ± 91 min vs. 579 ± 94 min, respectively (Table 3.6). In contrast, Puyau cutpoints revealed significantly (P<0.05) more sedentary time per day when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs, respectively (Table 3.6). In addition, Evenson cutpoints revealed more sedentary time per day when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs, respectively (Table 3.6). There was no significant (P = 0.07) difference for Sirard cutpoints across all the 3 epochs evaluated. However, Sirard cutpoints reported significantly (P<0.05) more sedentary time compared to Reilly, Puyau and Evenson cutpoints using 15 s, 30 s and 60 s epoch (Table 3.6). Similarly, for adolescents, choice of epoch had a significant effect on sedentary time (F (1.7, 277) = 843.8, *P<0.05*. *Post hoc* analysis revealed significantly (P<0.05) less sedentary time per day using Reilly cutpoints when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs: 621 ± 106 min vs. 619 ± 108 min and 621 ± 106 min vs. 619 ±

109 min, respectively (Table 3.7). In contrast, Puyau cutpoints revealed significantly ($P<0.05$) more sedentary time per day when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs, respectively (Table 3.7). In addition, Evenson cutpoints revealed more sedentary time per day when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs, respectively (Table 3.7). On the other hand, there was no significant ($P = 0.10$) difference for Sirard cutpoints across all the 3 epochs evaluated. However, Sirard cutpoints reported significantly ($P<0.05$) more sedentary time compared to Reilly, Puyau and Evenson cutpoints using 15 s, 30 s and 60 s epoch (Table 3.7).

Moderate to vigorous intensity physical activity (MVPA): Choice of epoch and cutpoints had a significant effect on MVPA time (Kruskal Wallis, $P<0.001$) for children. Mann Whitney U analysis revealed significantly more MVPA time using Pate, Sirard, Puyau and Evenson cutpoints when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs (Table 3.6). When comparing different cutpoints, the Pate cutpoints reported significantly more MVPA time compared to Sirard, Puyau and Evenson cutpoints ($P<0.05$) across all the epochs (Table 3.6). Similarly, for adolescents, choice of epoch and cutpoints had a significant effect on MVPA time (Kruskal Wallis, $P<0.05$). Mann Whitney U analysis revealed significantly more MVPA time using Pate, Sirard, Puyau and Evenson cutpoints when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs (Table 3.7). When comparing different cutpoints, the Pate cutpoints reported significantly more MVPA time compared to Sirard, Puyau and Evenson cutpoints ($P<0.05$) across all the epochs (Table 3.7).

Table 3.6: Time in minutes in sedentary and MVPA across all epochs as determined using Sirard, Puyau, Reilly, Pate and Evenson cutpoints in children. Data presented as mean (SD) for sedentary time and median [range] for MVPA.

Sedentary	15	30	60
Sirard	$616 \pm 94^{c,h,i}$	$620 \pm 95^{c,h,i}$	$624 \pm 97^{c,h,i}$
Puyau	$548 \pm 90^{c,I,k}$	$541 \pm 91^{a,c,I,k}$	$536 \pm 92^{b,c,i,k}$
Reilly	$570 \pm 91^{g,h,i,k}$	$579 \pm 93^{a,g,h,i,k}$	$579 \pm 94^{b,g,h,I,k}$
Evenson	$387 \pm 56^{c,h}$	$345 \pm 51^{a,c,h}$	$305 \pm 59^{b,c,h}$

MVPA	15	30	60
Sirard	18 [1 - 80]^{e,f,j}	12 [0 - 70]^{a,e,f,j}	9 [0 - 71]^{b,e,f,j}
Puyau	24 [1 - 100]^{d,e,g,j}	18 [0 - 93]^{a,d,e,g,j}	13 [0 - 84]^{b,d,e,g,j}
Pate	78 [4 - 197]^{d,f,g,j}	72 [3 - 202]^{a,d,f,g,j}	66 [1 - 201]^{b,d,f,g,j}
Evenson	53 [19 - 98]^{d,e,f,g,}	48 [15 - 81]^{a,d,e,f,g}	40 [10 - 83]^{b,d,e,f,g}

a and b: indicate significant difference from 15 s and 30 s epoch, respectively.

c: indicates significant difference between Reilly vs. Sirard, Puyau and Evenson cutpoints.

d: indicates significant difference between Sirard vs. Puyau, Pate, and Evenson cutpoints.

e: indicates significant difference between Pate vs. Sirard Puyau and Evenson cutpoints.

f: indicates significant difference between Puyau vs. Sirard and Pate and Evenson cutpoints.

g: indicates significant difference between Sirard vs. Pate, Puyau and Evenson cutpoints.

h: indicates significant difference between Puyau vs. Sirard and Reilly and Evenson cutpoints.

i: indicates significant difference between Evenson vs. Sirard, Puyau and Reilly cutpoints

j: indicates significant difference between Evenson vs. Sirard, Puyau and Pate cutpoints

k: indicates significant difference between Sirard vs. Puyau, Reilly and Evenson cutpoints

Table 3.7: Time in minutes in sedentary and MVPA across all epochs as determined using Sirard, Puyau, Reilly, Pate and Evenson cutpoints in adolescent. Data presented as mean (SD) for sedentary time and median [range] for MVPA.

Sedentary	15	30	60
Sirard	655 ± 102[c,h,i]	658 ± 103[c,h,i]	662 ± 104[c,h,i]
Puyau	655 ± 102[c,i,k]	658 ± 103[a,c,i,k]	662 ± 104[b,c,i,k]
Reilly	621±106[h,i,k]	619±108[a,h,i,k]	619±109[b,h,i,k]
Evenson	376 ± 69[c,h,k]	350 ± 75[a,c,h,k]	325 ± 72[b,c,h,k]
MVPA	**15**	**30**	**60**
Sirard	18 [1 - 80][e,f,j]	12 [0 - 70][a,e,f,j]	9 [0 - 71][b,e,f,j]
Puyau	24 [1 - 100][d,e,g,j]	18 [0 - 93][a,d,e,g,j]	13 [0 - 84][b,d,e,g,j]
Pate	107 [33 - 264][d,f,g,j]	100 [32 - 261][a,d,f,g,j]	96 [26 - 257][b,d,f,g,j]
Evenson	85 [28 - 223][d,e,f,g,]	78 [22 - 222][a,d,e,f,g]	66 [17 - 205][b,d,e,f,g]

a and b: indicate significant difference from 15 s and 30 s epoch, respectively.

c: indicates significant difference between Reilly vs. Sirard, Puyau and Evenson cutpoints.

d: indicates significant difference between Sirard vs. Puyau, Pate and Evenson cutpoints.

e: indicates significant difference between Pate vs. Sirard Puyau and Evenson cutpoints.

f: indicates significant difference between Puyau vs. Sirard and Pate and Evenson cutpoints.

g: indicates significant difference between Sirard vs. Pate, Puyau and Evenson cutpoints.

h: indicates significant difference between Puyau vs. Sirard and Reilly and Evenson cutpoints.

i: indicates significant difference between Evenson vs. Sirard, Puyau and Reilly cutpoints

j: indicates significant difference between Evenson vs. Sirard, Puyau and Pate cutpoints

k: indicates significant difference between Sirard vs. Puyau, Reilly and Evenson cutpoints

3.4 Discussion:

In the current study, subject compliance declined with increasing monitoring period for both child and adolescent samples (Table 3.3) which has also been observed in previous studies (Màsse *et al.*, 2005, Colley *et al.*, 2010) i.e. a decline in protocol adherence with extensive monitoring which in turn may compromise the reliability of accelerometer outcome variables. Several strategies have been proposed to promote protocol adherence such as asking study participants to complete an activity monitoring log and provision of incentives contingent on compliance such as money or gifts (Trost *et al.*, 2005). However, ethical issues may arise on inducing subject participation through monetary gain. Thus, subject compliance remains a significant practical issue in accelerometry since it is critical in achieving good reliability. Seasonality due to school terms, school holidays and climate is another important factor to consider when estimating habitual physical activity and sedentary time in children and adolescents (Corder *et al.*, 2008). Therefore, determining reliability estimates for accelerometer outcome variables is necessary to assess the robustness of these variables over several days of monitoring in specific populations. Reliability analysis of accelerometer outcome variables indicated that a minimum of 6 h for 7 - 9 days of monitoring and including at least 1 weekend day was required to achieve 80% reliability in the children (Table 3.4). The predicted number of days required to reliably assess physical activity and sedentary time in the present study was somewhat higher than the 3 - 5 days previously suggested for children (Reilly *et al.*, 2008). However, compliance rates tend to fall with increasing days of monitoring (Table 3.3) and therefore, there needs to be an optimal balance between retaining sufficient subject numbers for longitudinal analysis and achieving good reliability which ranges from 70 - 90% (Corder *et al.*, 2008).

Reliability analysis of accelerometer outcome variables in adolescents indicated that a minimum of 6 h for 5 days of monitoring and including at least 1 weekend day was sufficient to achieve 80% reliability (Table 3.4). The calculated ICC values in the current study were proximate to those observed by Mattocks and colleagues (2008a) in a large epidemiological study. Furthermore, the predicted number of days which should include at least 1 weekend day required to reliably monitor physical activity and sedentary behaviour in adolescents is consistent with the previously recommended 4 - 9 days of monitoring protocol previously recommended for adolescents (Trost *et al.*, 2005, Corder *et al.*, 2008). The finding that wearing a monitor for longer hours reduces reliability (Table 3.4) is counterintuitive, as one would expect that longer monitoring periods would produce better reliability. However, this is based on the assumption that subject compliance remains constant throughout the monitoring period which is not the case (Table 3.4). Penpraze et al. (2006) found that the estimated reliability for 7 consecutive days of measurement for 13 h/d was only 52% compared with 80% for 10 h/d of monitoring per day in children. These authors also found that the reliability of physical activity measured remained relatively stable from as little as 3 h/d up to 10 h/d of monitoring, however reliability declined for >10 h/d of monitoring (Penpraze *et al.,* 2006). Thus, it can be concluded that with constant or even reduced daily periods of monitoring, higher reliabilities are possible if the number of days of monitoring is increased (Penpraze *et al.,* 2006). Therefore, the number of days of monitoring is more important to reliability than the number of hours which is consistent with the findings of this study.

A calculation of ICC is promising in attempting to accurately and reliably assess physical activity and sedentary behaviour levels across a variety of samples and measurement protocols. However, applying any of these target number of days to all studies of physical activity and sedentary behaviour in children and adolescents may have inherent limitations (Baranowski & De Moor, 2000). The sample specific nature of the ICC has been demonstrated in a number of physical activity studies (Baranowski & De Moor, 2000, Trost *et al*., 2000, Baranowski *et al.,* 2008) which is consistent with the variable ICC values observed in the child and adolescent samples in this study. This is because the magnitudes of intra and interindividual variances in physical activity are specific to the sample in which they are collected, and the factors that influenced physical activity in the days that were sampled in the monitoring period. Furthermore, the Spearman–Brown formula assumes the ICC remains the same when additional monitoring days are added which may not be the case. For instance, if days with smaller day to day variation in physical activity are sampled (e.g. only weekdays monitored), the observed intraindividual variability is likely to be smaller, resulting in a higher ICC. Moreover, if a sample containing participants with greater interindividual variation in physical activity was used, the ICC would be increased (Baranowski & De Moor, 2000, Ridley *et al*., 2009). Furthermore, accelerometer data may not be measured in uniform periods each day (Catellier *et al*., 2005). Therefore, the most important decision rule in accelerometry is to determine what constitutes a valid day. A day varies for individuals in different age groups and may also vary depending on whether physical activity is being measured on a weekday or a weekend day (Catellier *et al*., 2005, Màsse *et al*., 2005, McClain & Tudor-Locke, 2009). The accelerometer data reduction decision rules and strategies to improve reliability of data collection highlighted above may therefore introduce selection bias, either due to days dropped or days retained, both of which would result in inaccurate representation of habitual physical activity (Catellier *et al*., 2005, Baranowski *et al.,* 2008). In addition, measurement error may result from large intraindividual variability in daily physical activity and sedentary behaviour habits, making it difficult to measure the true accelerometer outcome variables (Baranowski & De Moor, 2000, Baranowski *et al.,* 2008). Moreover, the variation in daily activity patterns caused by weekend/weekday and seasonal effects result in systematic variations, as all subjects experience weekdays, weekends and changes in season, no matter how a sample is selected (Ridley *et al*., 2009). Therefore, activity measured on a single day is likely to be a poor estimate of activity over a week, a month or a year (Ridley *et al*., 2009). In addition, accelerometer data screening needs to be carried out to ensure biological plausibility and to eliminate/minimize the influence of spurious data (such as consecutive zeroes inconsistent with monitor wear) on reported outcome variables (Cliff *et al*., 2009). This is identified either using activity diaries, assumption of sleeping time or identifying data segments of continuous inactivity surpassing a length beyond which it is deemed unlikely that the monitor could have been worn, e.g., 10 - 60 min (Treuth *et al*., 2003). Periods of 20 min or more consecutive zero counts are reported to be inconsistent with monitor wear (Treuth *et al*., 2003).

Accelerometer data inclusion criteria are mainly determined by a balance between retaining sufficient samples sizes for longitudinal studies and good reliability in a given study population (Corder *et al*., 2008, Mattocks *et al*., 2008b) and are therefore a potential source of significant variation within and between study populations (Màsse *et al*., 2005). Thus, comparisons between studies are not only difficult but could also result in significant inconsistencies in reported sedentary time and physical activity outcome variables within a given study population (Màsse *et al*., 2005, Colley *et al*., 2010) as reported in this study.

In support of the current debate on the most appropriate epoch length for accurate assessment of sedentary time and physical activity in paediatric and adolescent studies, the present findings indicate that lower epoch (15 s) durations reveal significantly less sedentary time using Sirard and Reilly cutpoints and more MVPA time across all the four cutpoints evaluated in children and adolescents. This is consistent with the finding that high frequency sampling of physical activity appears more accurate and reliable in quantifying physical activity in children (Dorsey *et al*., 2009). In children, choice of epoch had a significant effect on the time spent engaged in sedentary activity (Table 3.6) with shorter (15 s epoch) vs. longer (60 s epoch) resulting in significantly lower sedentary time using both Sirard and Reilly cutpoints (Table 3.6). However, using Puyau cutpoint the opposite was found, with significantly higher sedentary time for a 15 s vs. 60 s epoch (Table 3.6). This finding is in contrast to a previous study by Reilly *et al*. (2008) who reported no significant epoch effect on classification of sedentary time. However, the present study involved higher subject numbers compared to the previous study (96 vs. 32 subjects) which may explain this discrepancy. Similarly, for adolescents, epoch selection had a significant effect on the time spent engaged in sedentary activities (Table 3.7). This similarly contradicts the Reilly *et al*., 2008 study. Furthermore, choice of epoch has more pronounced effects in more active cohorts, for instance using Evenson cutpoints, 15 s epoch reported 19 min more MVPA time in adolescents compared to 60 s epoch. In children on the other hand, 15 s epoch reported 13 min more MVPA compared to 60 s epoch (Table 3.6, Table 3.7).

Epoch selection had a significant influence on reported MVPA time using all four cutpoints for both children and adolescents, with approximately 10 - 19 min more MVPA time with 15 s epoch compared to 60 s epoch (Table 3.6, Table 3.7). These findings may be physiologically significant. The lower Pate cutpoint threshold for moderate intensity activity resulted in subjects being classified as more active than the higher moderate intensity physical activity thresholds such as the Puyau and Sirard cutpoints. Previous studies reported similar trends, that is, a decrease in reported MVPA time as epoch length increased (Nilsson *et al*., 2002, Reilly *et al*., 2008). In contrast, Edwardson & Gorely (2010) reported the opposite trend in their adolescent sample, where shorter epoch durations were associated with less MVPA time. However, these authors also reported a non significant increase in MVPA time with decrease in epoch length in younger children aged 7 - 11 y. These authors suggested that choice of epoch is less important if the aim of a study is to measure MVPA but in this study epoch length was significantly associated with time spent in MVPA in both child and adolescent samples depending on which cutpoint was selected. This discrepancy between studies may be due to the use of age adjusted cutpoints in the Edwardson & Gorely (2010) study, or due to sample specific differences between these two studies. Differences in published activity thresholds are too large to allow meaningful comparison between studies (Guinhoya *et al*., 2006). In contrast, the accelerometer outcome variable CPM was relatively independent of cutpoint selection and choice of epoch in both children and adolescent cohorts. Therefore, this measure of physical activity may allow greater ease of comparison between studies.

In summary, this study has clearly demonstrated the effect that both epoch and cutpoints have on sedentary and MVPA classification in young children and adolescents. Even though it is clear from the results presented that cutpoints and epoch have a significant effect on reported sedentary time and MVPA levels, the actual physiological significance of the modest differences observed when sampling at 15 s vs. 60 s epoch across the different cutpoints has yet to be determined (Roberts & Freedson, 2007). For example, it is unclear if these relatively small differences in MVPA measured using shorter epochs actually contribute to the suggested health benefits of achieving 60 min MVPA per day (Cavill *et al*., 2001, CMO, 2011). Epoch and cutpoints have a significant effect on sedentary and MVPA classification, but the effect varies depending on the cutpoints and epoch selected. It is emphasized that for ease of comparison between studies, consensus should be achieved on the choice of epoch and cutpoints used to assess physical activity and sedentary time in children and adolescents. The existence of multiple sets of intensity related cutpoints for children and adolescents has significantly hindered research efforts to quantify, understand, and intervene on children and adolescent physical activity behaviour. Indeed, the lack of consensus on cutpoint selection and the widespread practice of deriving new calibration equations/cutpoints for a single population group or single study has created a cutpoint conundrum (Trost, 2007). In a recent study, Trost and colleagues (2011) examined the classification accuracy of five sets of independently developed ActiGraph cutpoints using energy expenditure measured by indirect calorimetry as a criterion reference standard in children and adolescents. These authors reported that only the Evenson cutpoints provided acceptable classification accuracy for sedentary behavior and physical activity intensity among children and adolescents (Trost *et al*., 2011). Therefore, the Evenson cutpoint expressed in 15 s epoch is recommended for future physical activity studies using ActiGraph accelerometers in children and adolescents. Furthermore, at least 6 h for 7 - 9 days of monitoring and including at least 1 weekend day would appear to be necessary to assess reliably physical activity and sedentary time in young children. For adolescent studies on the other hand, at least 6 h for 5 days of monitoring and including at least 1 weekend day is necessary to assess reliably physical activity.

Chapter 4

4.0: Validity of uniaxial and triaxial accelerometer outputs during structured activities in adolescents:

4.1 Introduction:

Accelerometry is a useful technique in the assessment of free living physical activity and may also be used to predict energy expenditure and $\dot{V}O_2$ (Welk *et al*., 2000, Chen & Bassett, 2005, Fudge *et al*. 2007) or METs (Freedson *et al*., 1998), which can be computed by normalizing energy expenditure to BMR. The assessment of energy expenditure in free living subjects is central to a complete understanding of the aetiology of obesity, malnutrition, coronary heart disease, and osteoporosis (Schoeller & Racette, 1990, Jakicic & Otto, 2005). Therefore, the translation of arbitrary accelerometer counts to caloric expenditure benefits epidemiological studies of disease and physical activity because energy expenditure is theorized to be physiologically related to mechanisms of diabetes, cancer, and other chronic conditions (Troiano, 2006). Several accelerometer models have been subjected to validation and calibration against DLW or indirect calorimetry as the criterion measure resulting in a number of different energy expenditure prediction equations (Rothney *et al*., 2008a, Rothney *et al*., 2008b). Some of these equations have been further used to develop cutpoints, which serve to discriminate physical activity intensities. Unfortunately, all of these equations are specific to a particular accelerometer device (Rothney *et al*., 2008a, Rothney *et al*., 2008b) and may also be sample/population specific. In spite of this, accelerometry has become the method of choice for objective assessment of habitual physical activity in children and adolescents (Trost, 2001).

Irrespective of accelerometer device, the raw acceleration signal is usually measured using proprietary algorithms that translate it into an arbitrary unit called accelerometer count which is not comparable across accelerometer monitor brands and probably across accelerometer generations. As such, there is need to convert counts to more meaningful indicators of physical exertion (Corder *et al.*, 2008). Therefore, the current research focus is aimed at validating accelerometers in field settings and across different populations. Furthermore, the development of novel and technically improved devices implies a need to validate these devices. This is typically done using mechanical oscillators (Rothney *et al.,* 2008b) or structured activities in humans (Kozey *et al.*, 2010c) and evaluating accelerometer outputs generated from theoretically identical acceleration signals.

The ActiGraph™ line of accelerometers is among the most frequently used activity monitors in the physical activity research field (Rothney *et al.,* 2008b). Several generations of these accelerometers are concurrently is use in paediatric and adolescent studies (Rothney *et al.,* 2008b). Extensive work has been dedicated to the development of correlations between ActiGraph data and physiological criteria, such as $\dot{V}O_2$ during walking and jogging in a laboratory setting (Melanson *et al.*, 1995, Freedson *et al.*, 1998). Researchers have expanded these studies to include field data using portable indirect calorimeters as the validation criterion (Bassett *et al.*, 2000, Crouter *et al.*, 2006, Hendelman *et al.*, 2000, Welk, 2005, Pober *et al.*, 2006). Despite the widespread use of the ActiGraph (De Vries *et al.,* 2006), considerable uncertainty exists about how to relate its output to $\dot{V}O_2$, energy expenditure or estimates of physical activity intensity (Trost, 2001) or how to relate outputs across different accelerometer generations, since only the earlier generations of these accelerometers have been validated in children and adolescents against criterion measures (Freedson *et al.,* 1998, Brage *et al.,* 2003, Welk, 2005). Recent studies indicate that outputs from different generations of ActiGraph accelerometers may be comparable and are valid in the assessment of physical activity (Kozey *et al.,* 2010c, John & Bassett, 2010). However, the validity of the latest ActiTrainer and GT3X (ActiGraph, LLC, Pensacola, FL, USA) accelerometers remains to be determined. The purpose of this study therefore was firstly; to determine the validity of uniaxial ActiTrainer (longitudinal axis) and triaxial GT3X (i.e. sum of X, Y and Z axes) accelerometer outputs during structured activities in adolescents and secondly, to determine the predicitve validity of ActiTrainer outputs against DLW determined TEE in adolescents.

4.2 Material and methods:

4.2.1 Subjects:

Volunteers were recruited and screened as detailed in section 2.1.5. Specific inclusion criteria are defined in section 2.1.6. As such, 30 adolescents were recruited from a rural Kenyan school, in the District of Nandi, Kenya. However, only 22 adolescents (11 females and 11 males) had complete accelerometer, HR and $\dot{V}O_2$ data (Table 4.1) and were used in the final data analyses.

This study design involved the portable K4b^2 gas analyser which was deemed to be too cumbersome for use in the child sample, furthermore the incremental running protocol was considered to be unsuitable for young children. Therefore, validation of accelerometers against indirect calorimetry was not replicated in the child sample.

4.2.2: Validation of the K4b^2 gas analyser:

The portable K4b^2 (COSMED, Rome, Italy) was designed to measure breath by breath pulmonary gas exchange in the field. In a previous study, Fudge and colleagues determined that an older generation of the K4b^2 was valid for measuring $\dot{V}O_2$ during treadmill running indoors, however during outdoor running the K4b^2 systematically underestimated $\dot{V}O_2$ (Fudge *et al.*, 2008). An automated periodical recalibration was introduced in the later generation of the K4b^2 in an attempt to correct the systematic underestimation in $\dot{V}O_2$ previously reported (Fudge *et al.* 2008). A recent study by Ross *et al.* (2011) validated the latest generation of the K4b^2 (which was used in the current study) in 19 Caucasian endurance trained individuals and 30 Kenyan adolescents. Subjects completed maximal incremental running tests. Tests involved 3 min exercise bouts at running speeds between 8 to 22 km/h in 2 km/h increments. Gas exchange parameters were recorded throughout. The Douglas bag method was used as the criterion for comparison. K4b^2 measurements were not significantly different for $\dot{V}O_2$ throughout all submaximal and maximal running speeds when compared to Douglas bag method: corresponding to a K4b^2 mean bias: -31 ml/min with limits of agreement -430 to 492 ml/min for all running speeds. K4b^2 test retest reliability was also demonstrated with no significant differences found in all measured gas exchange variables throughout all submaximal and maximal running speeds: corresponding to a K4b^2 mean bias -31 ml/min with limits of agreement -535 to 473 ml/min for all running speeds. This study concluded that the introduction of an automated calibration in the $K4b^2$ device improved its validity and reliability for assessment of $\dot{V}O_2$ during submaximal and maximal running velocities outdoors (Ross *et al.,* 2011).

4.2.3 Protocol:

Subjects performed structured activities while simultaneously fitted with two commercially available accelerometers, the uniaxial ActiTrainer and the triaxial GT3X accelerometers worn on the right hip and secured by an elastic belt and a GPS unit (GPSports Team AMS Release 1.2.1.12, Fyshwick, Australia) which was used to obtain the exact running speeds. In addition, subjects were harnessed with the $K4b^2$ gas analyser and a HR transmitter belt (Suunto t6, Suunto Oy, Vantaa, Finland) on the chest to record HR continuously on the ActiTrainer accelerometer. The accelerometers were synchronized to begin data collection utilising an external timepiece. All the ActiGraph devices were calibrated by the manufacturer prior to commencement of the study. The accelerometers counts were expressed in a 15 s epoch. The $K4b^2$ was calibrated immediately before each test according to the manufacturer's guidelines. The guidelines consisted of a four step calibration process: ambient air calibration, reference gas calibration, delay calibration, and turbine calibration as detailed in section 2.2.1. The uniaxial and triaxial accelerometer outputs were determined over the same time frame as the $\dot{V}O_2$ and HR values and used to assess the validity of the accelerometer outputs and accuracy in physical activity assessment during the structured activities defined below. In addition, TEE was measured over 7 days in the same subjects under free-living conditions using the DLW technique (Speakman, 1997) as described in section 2.1.7. Baseline urine samples were collected before oral dosing with 0.15 g $^{2}H_2\,O^{18}$ and 0.12 g $^{2}H_2O$ per kg BM. The following day, subjects provided two timed urine samples (morning and evening). This was repeated over the 7 day urine collection period. Urine samples were stored at -4°C in cryogenically stable tubes until analysis by isotope ratio mass spectrometry. Samples were analyzed in duplicate for H_2O^{18} (Speakman et al. (1990)) and $^{2}H_2O$ (Speakman and Krol (2005)). Carbon dioxide production rate was estimated from the differential disappearance of the 2 isotopes based on multi-point elimination curves (Djafarian et al. 2010) and the dilution space evaluated from the back-extrapolated intercept using equation A6 of Schoeller et al. (1986) and was converted to energy expenditure using the Weir equation (1949) assuming an average diet resulting in a respiratory exchange ratio of 0.85 (Black *et al.* 1986). Free-living daily physical activity levels and patterns were quantified using the ActiTrainer uni-axial accelerometer (ActiGraph LLC, Pensacola, FL, USA) with the recording epoch set at 15 s. Subjects wore the accelerometer for the same 7 consecutive days during school term time, during which DLW measurements took place. The mean accelerometer wear times was 807 ± 46 min, range [698 - 876 min] and mean nonwear time was 633 ± 46 min; range (562 - 742 min).

4.2.4 Structured activities:

For the purpose of comparing accelerometer outputs, lying quietly, sitting quietly, standing on the spot (were defined as sedentary activities). Jumping on the spot was used to define longitudinal acceleration with minimal anteroposterior and mediolateral displacements. Walking at 6 km/h, jogging 8 - 10 km/h, running at 12 - 14 km/h, and fast running 16 - 18 km/h (i.e. walking and running activities were selected to define acceleration in the longitudinal, anteroposterior and mediolateral vectors). These target activities represent body acceleration typical of free living sedentary and physical activities in adolescents. Furthermore, these activities were selected to provide graded increase from sedentary to vigorous intensity physical activity and to cover the broad range of locomotor activities that span the majority of the subjects' daily activities. All subjects performed these activities under the supervision of the investigators.

(a) Sedentary behaviour (lying, sitting, standing):

Subjects were instructed to lie quietly in a well ventilated and lighted room for 5 min. Subsequently, subjects were instructed to sit quietly for 5 min. Then subjects were instructed to stand still for 5 min. The $K4b^2$, HR belt and both accelerometers were worn during these sessions.

(b) Jumping on the spot:

Subjects were instructed to jump on the spot for 5 min while fitted with the $K4b^2$, HR belt and both accelerometers. Participants were provided with verbal encouragement to reach their maximum jump height throughout the test.

(c) Walking:

Subjects were fitted with the $K4b^2$ unit, the ActiTrainer and GT3X accelerometers, HR belt and a GPS unit. A researcher paced the subjects for 5 min at 6 km/h speed as they walked on level track field using a bicycle fitted with a digital speedometer (Timex Group, Connecticut, USA). The subjects were instructed to remain by the researcher's side throughout each test to maintain steady speed during the testing protocol. Throughout the testing process, each subject was verbally encouraged to maintain the pace and complete the 5 min of activity. The average GPS speed (4.9 ± 0.6 km/h) was used to classify walking.

(d) Discontinuous incremental running protocol:

Subjects were instructed to run at 8 km/h around a flat running track for 3 min interspersed with a 3 min walking interval at 6 km/h (active recovery), then subject ran at 10 km/h for 3 min with active recovery for 3 min. The running speed was discontinuously increased at 2 km/h interval to 18 km/h or to volitional exhaustion. Subjects were instructed to stay by the researchers' side who paced them using a bicycle fitted with a digital speedometer (Timex Group, Connecticut, USA) throughout each test speed. Throughout the tests, subjects were verbally encouraged to maintain the pace and complete the test activity. If the subject could not keep up with the pace, the test was stopped. All the subjects were able to complete the running protocol up to 16 km/h. However, none of the subjects could complete the running protocol at 18 km/h and therefore this speed was excluded from the analysis. Due to practical limitations subjects were unable to run at exact incremental running speeds specified in the protocol. Therefore, the average GPS speeds were used in the analysis. These were walking: 4.9 ± 0.6 km/h and jogging: 8 ± 0.3 km/h and 9.1 ± 0.5 km/h, running at 11.9 ± 0.3 km/h, 13.1 ± 0.5 km/h and 15.5 ± 0.4 km/h.

4.2.5 Statistical analysis:

Accelerometer, HR and K4b^2 data were averaged from the last 30 s of each test. Data were expressed as mean (standard deviation (SD) or median [range] following a Shapiro Wilk test for normality. Accelerometer outputs during all the structured activities were normally distributed except for sedentary activities. Simple linear regression analysis was used to examine the relationship between ActiTrainer and GT3X accelerometer outputs and $\dot{V}O_2$. Paired t-tests were used to test for differences in R^2 between ActiTrainer and GT3X outputs. In addition, the predictive validity of the ActiTrainer accelerometer was also examined against DLW determined energy expenditure. To get an approximate 24 h measure for TEE from the $\dot{V}O_2$ based measure, the average counts for the wear time for each subject was used to calculate the $\dot{V}O_2$ associated with this number of counts from the ActiTrainer regression line ($\dot{V}O_2$ = 0.024*ActiTrainer counts/15 s + 5.694) ml/kg/min. $\dot{V}O_2$ values were then transformed into caloric values assuming the standard constants (1 L O_2 = 4.8 kcal) (McLaughlin *et al.,* 2001). The energy expenditure values were then multiplied by the accelerometer wear time to get total energy expenditure for wear time. For non wear time, $\dot{V}O_2$ for sedentary activities were multiplied by non wear time to obtain total energy expenditure for non wear time. Finally, TEE was calculated as the sum of energy expenditure during wear and non wear times. The Bland & Altman method (1986) was used to compare the mean difference between the DLW determined TEE and the ActiTrainer predicted TEE, and 95% limits of agreement as the mean difference (1.96 SD). The mean error scores were graphically illustrated by a solid horizontal line and the limits of agreement (±1.96 SD from the mean) were shown as dashed horizontal lines. To estimate the degree of agreement, the difference between each set of two consecutive TEE measurements was determined, and the mean difference (bias) was calculated. The mean bias represents the degree of systematic difference between methods of measurement and was determined by summing the differences between paired measurements and dividing by the number of paired measurements. The limits of agreement (bias ±1.96 SD) defined the concordance interval, which encompassed 95% of the differences between each set of two consecutive TEE measurements. A tight prediction interval around zero is deemed comparable. Data points below zero signify an overestimation, while points above zero signify an underestimation (Bland & Altman, 1986). Paired t-tests were used to test if the differences in TEE were statistically significant. Statistical computations were performed using the software package SPSS, Version 17.0 (SPSS, inc., Chicago, IL, USA). Significance was declared at $P<0.05$.

4.3 Results:

4.3.1 Descriptive statistics:

Physical characteristics of study participants are shown in Table 4.1

Table 4.1: Physical characteristics of the study participants.

	All	Females	Males
Subject	22	11	11
Age (yrs)	14.1 ± 1.4	13.8 ± 1.5	14.3 ± 1.2
Weight (kg)	41.6 ± 9	42.8 ± 9.1	40.5 ± 9.2
Height (cm)	157.8 ± 10.9	156 ± 9.7	159.4 ± 12
BMI (kg/m^2)	16.5 ± 2.1	17.4 ± 2.4	15.7 ± 1.4

Both ActiTrainer and GT3X accelerometer outputs peaked during jumping (3858 ± 600 and 4824 ± 821 Cnts/15 s for ActiTrainer and GT3X counts respectively). All accelerometer outputs increased linearly from walking (4.9 ± 0.6 km/h) to running, but then plateaued for the ActiTrainer at running speeds of 9.1 ± 0.5 km/h (1728 ± 408 Cnts/15 s) (Table 4.2). Conversely, GT3X outputs did not plateau at the peak running speed of 15.5 ± 0.4 km/h (3573 ± 639 Cnts/ 15s) (Figure 4.1).

Figure 4.1: Accelerometer outputs, heart rate and oxygen uptake during structured activities in adolescents

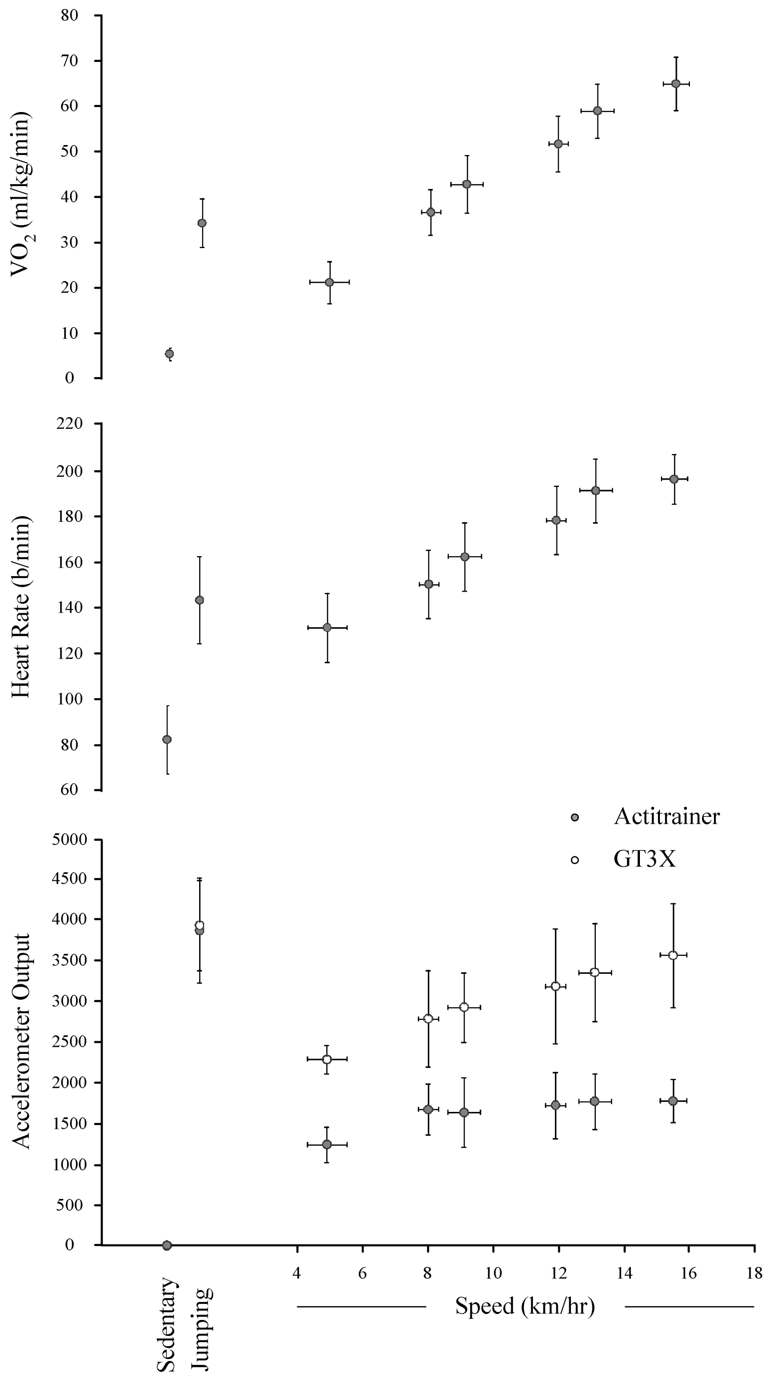


Table 4.2: $\dot{V}O_2$ **(ml/min/kg), HR and accelerometer outputs (Cnts/15s) during structured activities (Mean ± SD).**

Activity	$\dot{V}O_2$	**HR**	**ActiTrainer**	**GT3X**
Sedentary activities	5.3 ± 1.4	82 ± 15	0 [0 - 10]	0 [0 - 65]
Jumping	33.6 ± 5.7	143 ± 19	3879 ± 642	3943 ± 553
4.9 ± 0.6 km/h	21.0 ± 4.5	131 ± 15	1245 ± 219	2292 ± 178
8.0 ± 0.3 km/h	36.6 ± 5.2	150 ± 15	1677 ± 312	2793 ± 591
9.1 ± 0.5 km/h	42.8 ± 6.3	162 ± 15	1641 ± 423	2934 ± 429
11.9 ± 0.3	51.7 ± 6.1	178 ± 15	1728 ± 408	3192 ± 708
13.1 ± 0.5 km/h	59 ± 6	191 ± 14	1726 ± 344	3363 ± 606
15.5 ± 0.4 km/h	65 ± 5.9	196 ± 11	1782 ± 267	3573 ± 639

Mean ± SD
Median [range]
$\dot{V}O_2$ -Oxygen uptake
HR - Heart rate
Cnts/15s - Accelerometer counts per 15 s

4.3.2 Relation between accelerometer outputs, oxygen uptake, heart rate and physical activity intensity in adolescents:

In contrast to accelerometer outputs, mean $\dot{V}O_2$ increased systematically from lying down 5.3 ± 1.4 ml/kg/min to peak uptake of 64.5 ± 6.1 ml/kg at running speeds >15 km/h rather than during jumping (mean $\dot{V}O_2$ 33.6 ± 5.7) (Figure 4.1). Similarly, mean HR increased from sedentary activities to peak running speeds (82 ± 15 vs. 195 ± 11 beats/min) for sedentary vs. running at peak speed respectively (Figure 4.1). Furthermore, in all subject, the triaxial GT3X reported significantly (P<0.05) better fitting regression models compared to uniaxial ActiTrainer (Table

4.3). In addition, when jumping was excluded form the menu of activities, the fit of the regression models improved for both ActiTrainer and GT3X accelerometers (Table 4.4). As such, 40% of the variation in $\dot{V}O_2$ could be predicted by ActiTrainer (P < 0.05) compared to 55% for the GT3X when jumping was included in the structured activities protocol (Figure 4.2, Figure 4.3). On the other hand, when jumping was excluded, the fit of the regression improved to 76% and 80% for the ActiTrainer and GT3X accelerometers respectively (Figure 4.4, Figure 4.5). However, addition of any of the HR derivatives (mean HR, maximal HR, resting HR or ΔHR_{acc}) was not a significant predictor of $\dot{V}O_2$.

Table 4.3: The relation between ActiTrainer vs. GT3X accelerometer outputs and oxygen uptake during structured activities (sedentary, jumping, walking and running) in adolescents

Subject	ActiTrainer			GT3X		
	Gradient	**Intercept**	**R^2**	**Gradient**	**Intercept**	**R^2**
1	y = 0.008	19.42	0.31	y = 0.010	16.51	0.40
2	y = 0.008	14.05	0.49	y = 0.008	13.84	0.50
3	y = 0.013	15.15	0.45	y = 0.012	15.77	0.43
4	y = 0.012	18.39	0.45	y = 0.013	14.43	0.55
5	y = 0.010	15.52	0.42	y = 0.011	10.12	0.62
6	y = 0.009	14.56	0.44	y = 0.010	11.75	0.54
7	y = 0.012	15.49	0.54	y = 0.012	11.22	0.67
8	y = 0.011	13.71	0.52	y = 0.011	9.57	0.67
9	y = 0.020	9.06	0.61	y = 0.015	9.32	0.64
10	y = 0.011	16.52	0.34	y = 0.014	9.28	0.63
11	y = 0.010	21.11	0.18	y = 0.014	12.63	0.42
12	y = 0.012	20.38	0.27	y = 0.014	12.61	0.50
13	y = 0.013	17.52	0.31	y = 0.016	8.73	0.55
14	y = 0.015	12.24	0.58	y = 0.015	9.77	0.70
15	y = 0.012	16.22	0.35	y = 0.014	9.93	0.58
16	y = 0.011	16.87	0.33	y = 0.013	8.82	0.55
17	y = 0.014	14.64	0.43	y = 0.014	8.33	0.61
18	y = 0.013	13.67	0.41	y = 0.014	11.67	0.58
19	y = 0.011	15.71	0.29	y = 0.012	12.84	0.41
20	y = 0.008	16.54	0.35	y = 0.009	11.73	0.49
21	y = 0.021	13.93	0.43	y = 0.021	11.38	0.54
22	y = 0.024	10.18	0.39	y = 0.019	12.13	0.52
Mean	**y = 0.013**	**15.49**	**0.40**	**y = 0.013**	**11.47**	**0.55**

Table 4.4: The relation between ActiTrainer vs. GT3X accelerometer outputs and oxygen uptake during structured activities (sedentary, walking and running)†.

Subject	ActiTrainer			GT3X		
	Gradient	**Intercept**	**R^2**	**Gradient**	**Intercept**	**R^2**
1	y = 0.022	4.67	0.69	y = 0.022	3.75	0.74
2	y = 0.015	5.78	0.78	y = 0.015	4.43	0.82
3	y = 0.024	4.62	0.80	y = 0.024	3.78	0.82
4	y = 0.026	4.73	0.86	y = 0.022	5.21	0.83
5	y = 0.022	4.74	0.85	y = 0.018	2.87	0.72
6	y = 0.020	4.01	0.88	y = 0.016	4.01	0.89
7	y = 0.023	4.72	0.94	y = 0.018	4.23	0.81
8	y = 0.020	5.73	0.83	y = 0.018	4.44	0.80
9	y = 0.028	5.33	0.87	y = 0.021	3.32	0.80
10	y = 0.023	8.04	0.65	y = 0.019	4.90	0.84
11	y = 0.031	6.91	0.53	y = 0.025	3.00	0.72
12	y = 0.030	7.94	0.64	y = 0.024	2.91	0.83
13	y = 0.027	6.54	0.70	y = 0.021	3.90	0.79
14	y = 0.025	4.42	0.81	y = 0.019	4.42	0.80
15	y = 0.025	6.46	0.80	y = 0.021	3.93	0.82
16	y = 0.027	6.94	0.70	y = 0.020	2.82	0.86
17	y = 0.029	5.23	0.71	y = 0.021	2.21	0.71
18	y = 0.028	7.51	0.77	y = 0.021	2.72	0.82
19	y = 0.025	5.54	0.72	y = 0.026	4.02	0.75
20	y = 0.023	5.17	0.72	y = 0.020	4.31	0.85
21	y = 0.023	4.82	0.72	y = 0.029	4.82	0.86
22	y = 0.023	5.41	0.76	y = 0.020	4.02	0.80
Mean	**y = 0.024**	**5.69**	**0.76**	**y = 0.021**	**3.82**	**0.80**

† - Excluding jumping on the spot

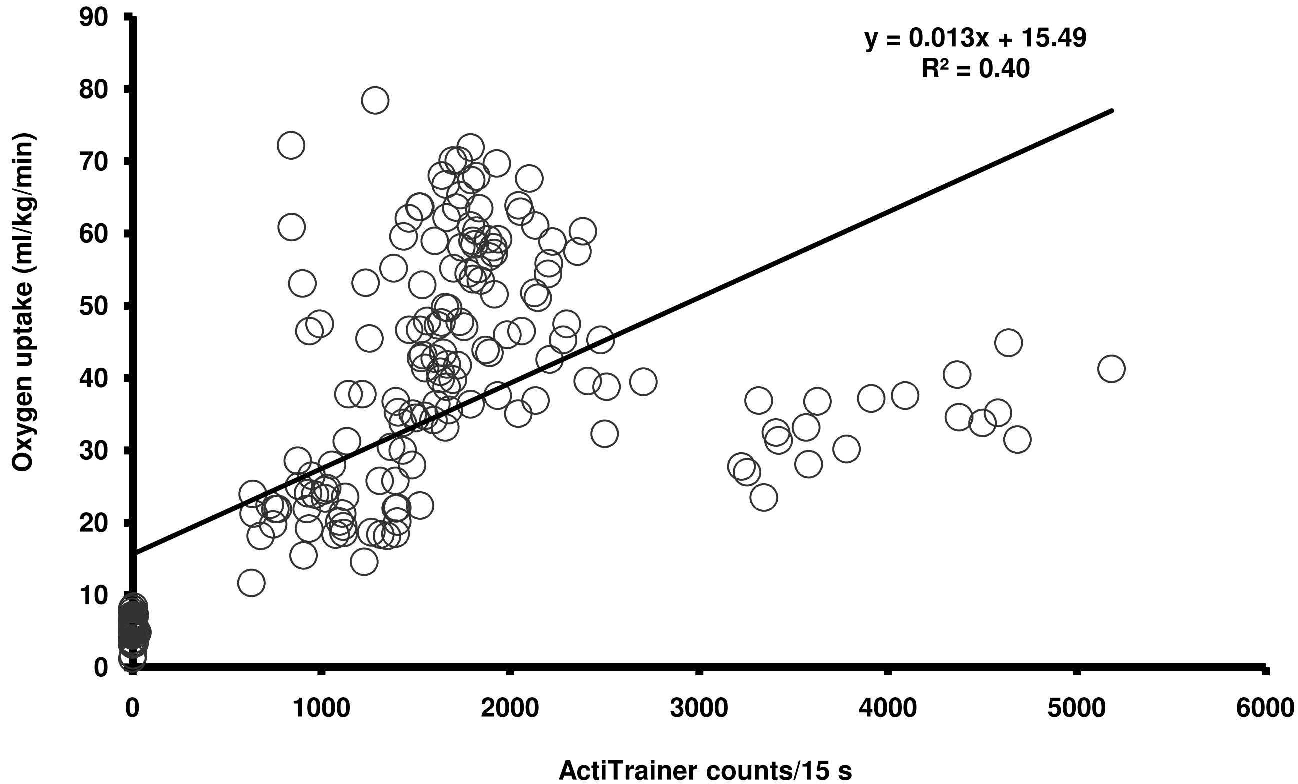


Figure 4.2: Relation between ActiTrainer counts and oxygen uptake in adolescents during structured activities (sedentary, jumping, walking and running)

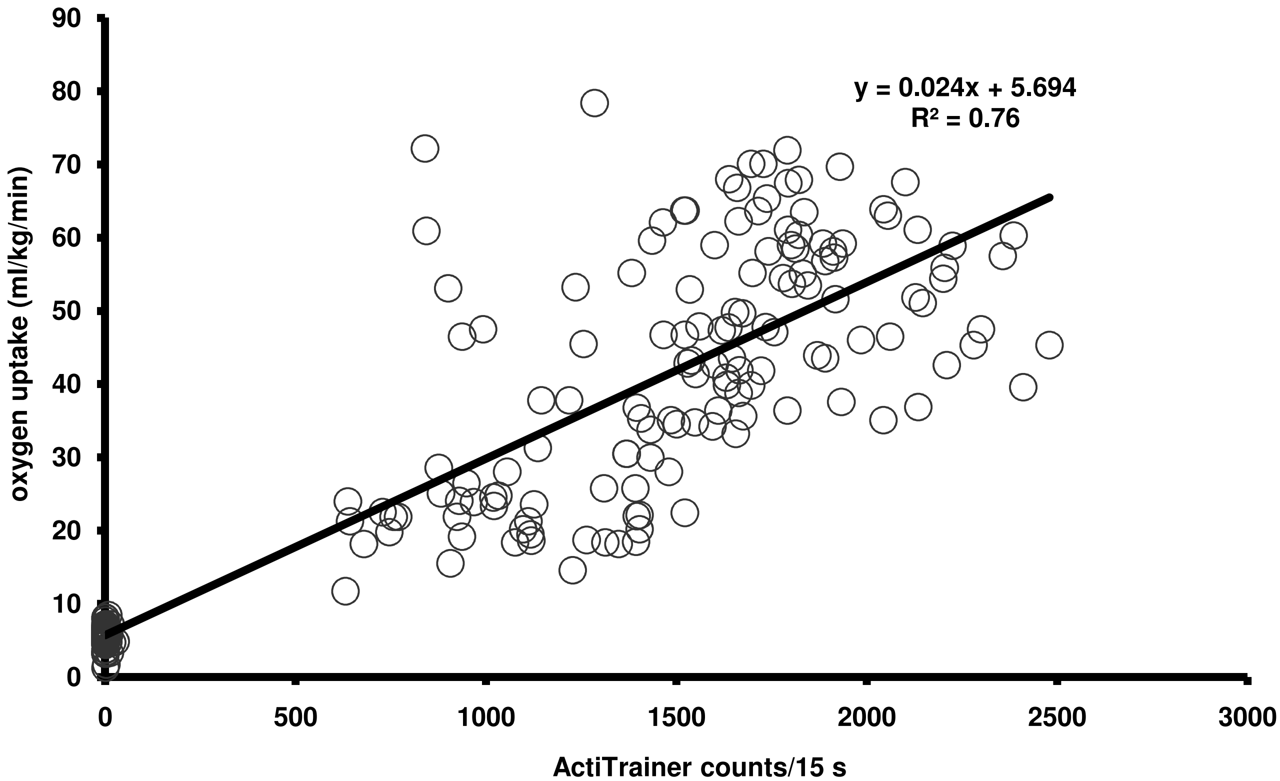


Figure 4.3: Relation between ActiTrainer counts and oxygen uptake in adolescents during structured activities (sedentary, walking and running)

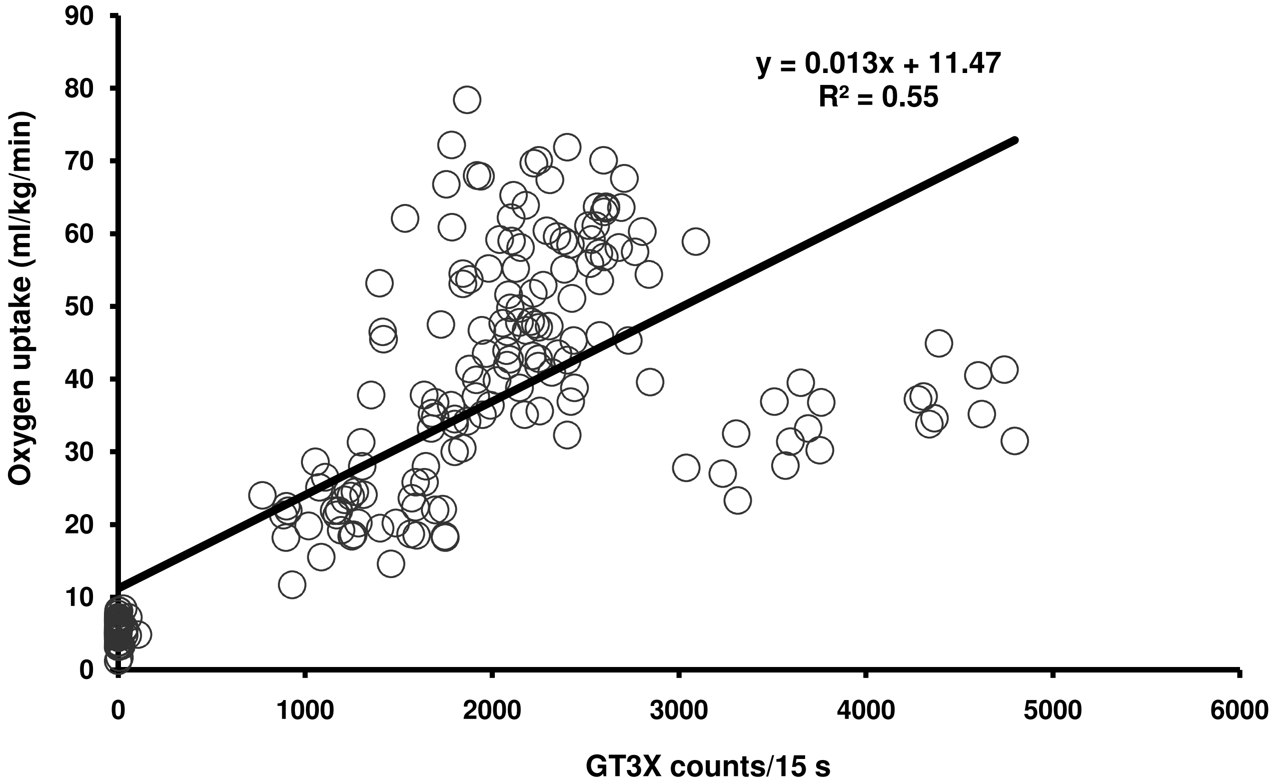


Figure 4.4: Relation between GT3X counts and oxygen uptake in adolescents during structured activities (sedentary, jumping, walking and running)

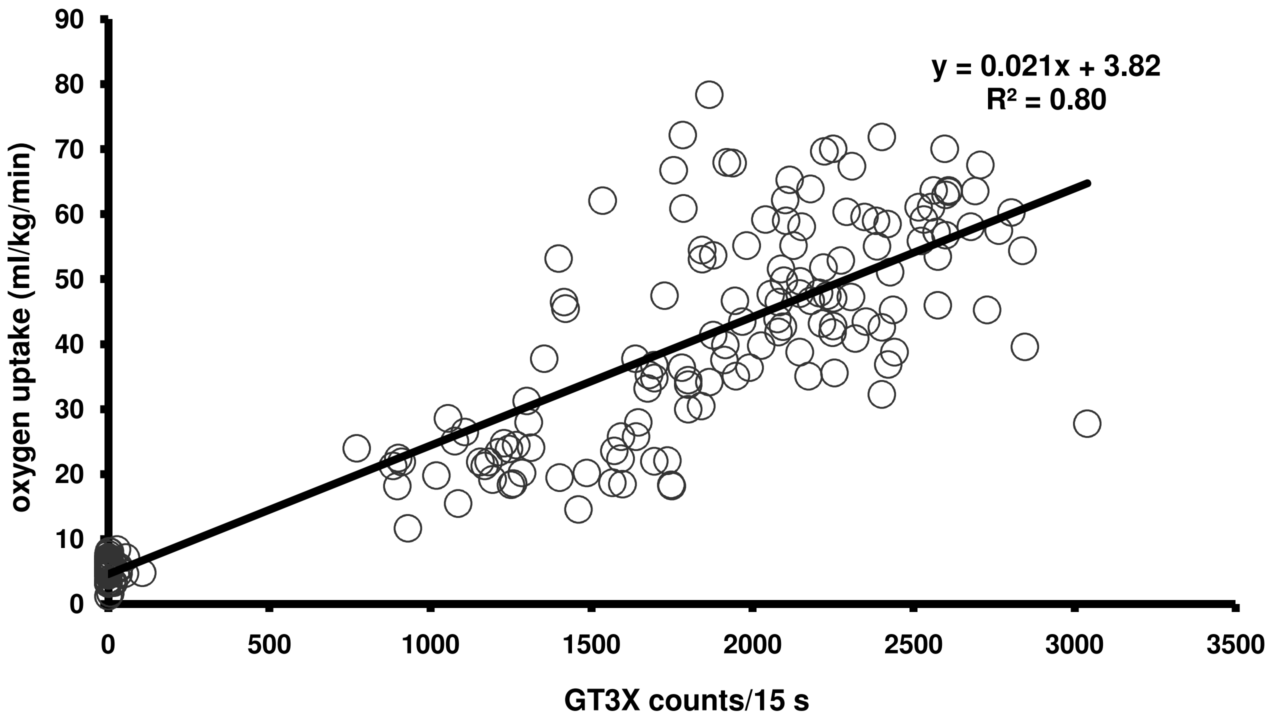


Figure 4.5: Relation between GT3X counts and oxygen uptake in adolescents during structured activities (sedentary, walking and running).

The predictive validity of the $\dot{V}O_2$ regression equation derived from the relationship between ActiTrainer outputs and $\dot{V}O_2$ during structured activites (i.e. $\dot{V}O_2$ = 0.024 (ActiTrainer counts) + 5.694)) was examined against DLW derived TEE during free living activites in the same subjects. The Bland-Altman plot for calculated TEE from DLW and predicted TEE from ActiTrainer counts had a mean bias of (-1.1) MJ/d and 95% prediction interval of (-6.8, 4.6) MJ/d. This difference was statistically significant (paired t-test, P=0.04; Figure 4.6).

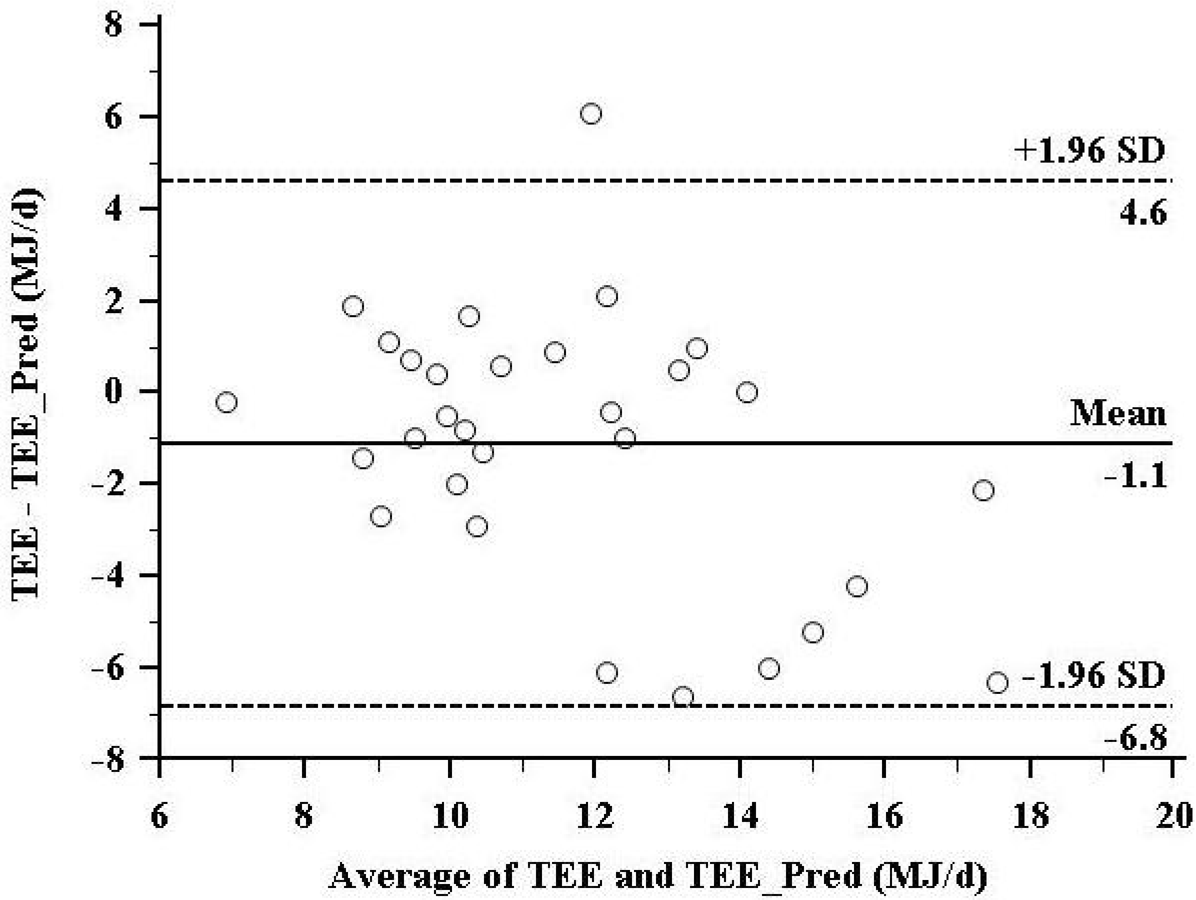


Figure 4.6: Bland-Altman plot showing difference against mean TEE (MJ/d) assessed by DLW (TEE) and ActiTrainer accelerometer (TEE_Pred). Error (TEE - TEE_Pred) TEE plotted against mean outputs of the two measurements. The mean error scores are illustrated by a solid horizontal line and the limits of agreement (±1.96 SD from the mean) are shown as dashed horizontal lines.

4.4 Discussion:

This study examined the validity of the novel ActiTrainer and GT3X accelerometer outputs during structured activities in adolescents. Accelerometer outputs were highest during jumping activity (Figure 4.1). However $\dot{V}O_2$ did not peak in tandem with accelerometer outputs during jumping. This finding is significant, since it may explain the lower fit of the accelerometer count prediction of $\dot{V}O_2$ (Figure 4.2, Figure 4.3). Furtheremore, exclusion of jumping from the protocol of activities significantly improved the fit of the regression models for both ActiTrainer and GT3X accelerometer (Figure 4.4, Figure 4.5). On the other hand, during the incremental running protocol, ActiTrainer and GT3X outputs rose with increasing running speeds and plateaued at an average speed of 9.1 ± 0.5 km/h for the ActiTrainer, which is lower than the levelling off speed of 10 - 12 km/h reported of the uniaxial CSA ActiGraph accelerometer (Fudge *et al*. 2007). The differences in peaking of uniaxial accelerometer outputs could be attributed to differences in running protocols. The Fudge *et al*. 2007 study was conducted using treadmill running compared to overland running protocol in this study. On the other hand, the triaxial GT3X accelerometer outputs did not level off at the peak running speed tested (Figure 4.1).

The peaking of the ActiGraph accelerometer outputs with increasing running speeds/acceleration has been observed in previous studies (Fudge *et al.,* 2007, Rothney *et al.,* 2008b, John & Bassett, 2010). This has been attributed to the device's amplitude and bandpass filtering limitations of these devices. However, to accurately estimate energy expenditure and $\dot{V}O_2$, accelerometer outputs must demonstrate a systematic increase whether (linear, curvilinear, exponential) in activity counts with increasing exercise intensity because energy expenditure/$\dot{V}O_2$ regression equations are based on the theory that activity counts increase with increasing intensity of exercise (John & Bassett, 2010). Therefore, it is expected that GT3X outputs may approximate better energy expenditure/$\dot{V}O_2$ associated with fast running activities since they demonstrate increased outputs at fast running speeds of (15.4 ± 0.5 km/h). GT3X accelerometer reported higher R^2 compared to uniaxial ActiTrainer (Figure 4.2, Figure 4.3). These differences, if biologically meaningful might reflect that GT3X has higher validity for measurement of physical activity even if it does not have any meaningful advantage over the ActiTrainer in prediction of $\dot{V}O_2$

The present study suggests that the novel ActiTrainer and GT3X ActiGraph accelerometers are valid for the assessment of $\dot{V}O_2$ associated with physical activity during structured activities in adolescents (R^2 = 76 - 80%) for the ActiTrainer and GT3X accelerometer respectively. Therefore, triaxial GT3X outputs was a better predictor of $\dot{V}O_2$ associated with structured activities compared to uniaxial ActiTrainer. These findings are broadly consistent with Plasqui and colleagues (2005) who reported that for a variety of activities, triaxial accelerometry is better at predicting energy expenditure than uniaxial. Thus, the use of the sum of all three axes improves the accuracy of energy expenditure/$\dot{V}O_2$ estimation, since acceleration recorded in the different axes can contribute to enhance the prediction, furthermore, energy expenditure could relate differently to acceleration components in other axes, depending on the type and nature of the activity (Chen & Bassett, 2005). A known limitation with uniaxial accelerometers is that they underestimate non ambulatory activities that do not involve vertical movement of the trunk (when waist mounted) such as cycling (Corder *et al.,* 2007a). Additionally, the plateau of uniaxial accelerometer outputs at fast running speed limits its usefulness in assessment of vigorous activities. However, use of the ActiTrainer outputs to predict energy expenditure during free living activities indicated improved validity with the difference in TEE derived form DLW and ActiTrainer outputs tending towards nonsignificance ($P<0.05$).

In summary, the GT3X triaxial accelerometer reports significantly higher accelerometer count outputs and better fitting regression models compared to the uniaxial ActiTrainer. These higher outputs may approximate better human movement which typically involves simultaneous movement in the vertical, mediolateral and anteroposterior planes and probably the $\dot{V}O_2$ associated with these movements. This is further supported by previous studies that indicate that physical activity related energy expenditure during sedentary as well as during physically intensive activities correlates better with the sum of body acceleration in all three dimensions rather than with the longitudinal displacement of the body, as monitored by uniaxial accelerometers (Plasqui *et al.,* 2005).

Chapter 5

5.0: Evaluation of free living energy expenditure using uniaxial accelerometry with heart rate monitoring versus triaxial accelerometry in children and adolescents:

5.1 Introduction:

Physical activity is a complex behaviour that varies with age, gender, season, week day and time of the day and is also influenced by biological, sociological, psychological and environmental factors (Bammann *et al*., 2006). Dietary changes may account for the secular trends in obesity (Appelhans et *al.,* 2012, de Castro *et al.,* 2012, Rouhani *et al.,* 2012, Steinsbekk *et al.,* 2012). For instance, in a large cross sectional study, the National Health and Nutrition Examination Surveys (1988 - 1994, n = 28, 663) reported that energy intake was the primary contributor of BMI (Song *et al.,* 2012). On the other hand, a decline in physical activity has been identified as a possible contributory factor in childhood obesity (Reilly, 2008) but is difficult to quantify precisely as few studies utilize the same methods of assessment and limited objective data exist (Bammann *et al*., 2006, Reilly, 2008). Physical activity is generally considered to be a central factor in the aetiology, prevention and treatment of childhood obesity (Jakicic & Otto, 2005) and thus, the quantification of energy expenditure and daily physical activity has gained considerable interest (Chen & Sun, 1997). However, accurate assessment of physical activity and sedentary behaviour, especially in children and adolescents remains a significant challenge (Bammann *et al*., 2006). Consequently, validated techniques of estimating habitual physical activity are needed to study the relationship between free living physical activity and obesity (Westerterp, 2009). These methods should be suitable to measure physical activity over periods long enough to be representative for normal daily life, with minimal discomfort to the subjects, and applicable to large study populations (Westerterp, 2009). The ability to accurately track energy expenditure using objective methods is crucial (Crouter *et al.,* 2006b), especially in studies that aim to assess the trends in energy expenditure over time. DLW is the only technique available to accurately measure TEE over prolonged periods in daily life (Bonomi *et al.,* 2010a). When this technique is combined with a measure of BMR, AEE or PAL can be calculated (Plasqui *et al.,* 2005). On the other hand, accelerometers provide a means by which researchers can examine the intensity, frequency, and duration of physical activity bouts that individuals are performing over extensive periods of time (Chen & Bassett, 2005). By validating an accelerometer against DLW derived energy expenditure, prediction models can be developed to predict TEE, AEE, or PAL from accelerometer counts and other physical characteristics, such as age, sex, height, and body mass (Plasqui *et al.,* 2005). Thus, improvement of the accuracy of accelerometers in predicting TEE, AEE, and PAL has been the focus of several studies (Chen & Sun, 1997, Plasqui & Westerterp, 2007, Bonomi *et al.,* 2009).

Uniaxial accelerometers measure accelerations in one axis (usually longitudinal), where as triaxial accelerometers measure accelerations in the anteriorposterior, mediolateral, and longitudinal directions (Chen & Bassett, 2005). Although uniaxial accelerometers are accurate in prediction of energy expenditure during walking, triaxial accelerometers are more suitable when a variety of different activities are involved (Bouten *et al.,* 1994, Welk *et al.,* 2000b, Leenders *et al.,* 2001, Plasqui *et al.,* 2005). A number of studies have reported improved accuracy to predict physical activity and energy expenditure when combining accelerometry with HR monitoring. For example, Heskell *et al*. (1993) reported an R^2 value improvement from 0.69 to 0.82 when arm motion, as assessed by accelerometry, was combined with HR monitoring during arm ergometer exercise. Subsequent validation studies have therefore explored the utility of combining accelerometry wih HR monitoring (Treuth *et al*., 1998, Rennie *et al*., 2000, Strath *et al*., 2001, Strath *et al*., 2002, Brage *et al*., 2003, Brage *et al*., 2004) but the results have been equivocal. Therefore combining accelereometry and HR needs further exploration.

The most widely used and extensively validated accelerometer for assessment of physical activity among children is the ActiGraph (ActiGraph LLC, Pensacola, FL, USA) accelerometer (Freedson *et al.,* 2005, Crouter *et al.,* 2006b). The ActiTrainer simultaneous collects activity counts and HR data. On the other hand, the capability of the 3DNX™ (BioTel Limited, Bristol, UK) accelerometer to predict energy expenditure in free living adult and adolescent cohorts has also been examined using the DLW method as a criterion measure (Carter *et al.,* 2008). These authors did not find any association between 3DNX outputs and PAL or AEE/kg, which are parameters directly related to physical activity (Carter *et al.,* 2008). The purpose of this study therefore, was to examine the validity of uniaxial accelerometry with HR monitoring vs. triaxial accelerometry against DLW criterion during free living activities in young children and adolescents.

5.2 Materials and methods:

5.2.1 Subjects:

49 children (25 female, 24 male) aged between 4 - 10 years (mean age 7 ± 2 years: Table 5.1) from the initial child sample (see section 2.1.3 for details) fulfilled the inclusion criteria for final data analysis i.e. at least 6 weekdays including at least 1 weekend day of valid recording of at least 360 min of continuous monitoring per day as determined in Chapter 3. Furthermore, subjects were required to have concurrent ActiTrainer, HR and 3DNX recording. Additionally, a subsample of 30 rural Kenyan adolescents (15 male, 15 female), mean age 14 ± 1 y form the initial adolescent cohort were recruited to participate in this study (see section 2.1.3 for details). All subjects fulfilled the inclusion criteria for data analysis i.e. at least 4 weekdays of monitoring, including 1 weekend day of valid recording of at least 360 min of continuous monitoring per day as determined in Chapter 3. In addition, subjects were required to have corresponding ActiTrainer and HR data.

5.2.2 Calorimetry:

Following the collection of a base line urine sample (Day 0), subjects drank a weighed amount of $^{2}H_2{}^{18}O$ resulting in an initial excess body water enrichment of 125 - 150 ppm for deuterium and 250 - 300 ppm for oxygen 18. Subsequent urine samples were collected from the second voiding in the morning and a subsequent voiding in the evening on Days 1, 4, and 8 for the child sample and daily for the adolescent sample as detailed in section 2.1.7. Urine samples were stored at -

4°C in cryogenically stable tubes until analysis by isotope ratio mass spectrometry. Samples were analyzed in duplicate for H_2O^{18} and ^2H_2O as detailed in section 2.1.7. Carbon dioxide production rate was estimated from the differential disappearance of the 2 isotopes based on the multipoint elimination curves and converted to energy expenditure as detailed in section 4.2.3 for both child and adolescent cohorts. In addition, the Schofield equations (1985) based on gender, age, height and weight were used to predict BMR for the child cohort as described in section 2.1.7.

5.2.3 Accelerometry:

Free living daily physical activity levels were objectively assessed using the uniaxial ActiTrainer and triaxial 3DNX accelerometers (see Section 2.1.8 for description) in the child sample. The adolescent sample wore the ActiTrainer only. Data were recorded continuously and downloaded to a computer after each experiment. In both devices, signal processing happens onboard, that is, the raw signal is first filtered by firmware, then integrated over the 15 s sampling period used in this study. For the child cohort, the data from ActiTrainer and 3DNX devices could not be exactly aligned because 3DNX accelerometer is started manually by pressing a button and therefore the exact start time cannot be preset unlike the case with the ActiTrainer. The resulting difficulty was not foreseen when the study was carried out: otherwise, one could have manually started the 3DNX exactly at the time when ActiTrainer was initialized. Retrospectively, the only thing that could be done was to summarize the data by longer periods (in this case, 30 min bouts), and keep only those periods that have complete data for both devices. Thus, 34545 h (in 30 min bouts) were initially matched. Subsequently, only 11791 h remained after deletion of non compliant periods (≥20 min consecutive zeroes as recommended by Treuth *et al*., (2003) (i.e. where some non wear time was detected by at least one of the monitors). Median wear time was 668 min, interquartile range: 76. On the other hand, median of wear time was 818 min, interquartile range: 72 for the adolescent sample. Accelerometer outputs included CPM and, time spent sedentary and in physical activities of different intensities based on Evenson cutpoints (2008) and HR data (minimal HR, maximal HR, mean HR). In addition a ΔHR_{acc} was also computed as described in section 2.1.9.

5.2.4 Physical activity assessment:

Subjects wore the accelerometers for 7 consecutive days between January and April 2009 during school term time simultaneous with the DLW measurements. Accelerometers were mounted on the right hip of each subject on the same elastic belt and adjusted to ensure close contact with the body. In addition, all subjects were fitted with a HR transmitter belt (Suunto t6, Suunto Oy, Vantaa, Finland) on the chest to record HR continuously. The subjects were required to wear the accelerometers and HR transmitter belts from the moment they woke up in the morning until bed time in the evening so that a full day of physical activity could be assessed. In addition, accelerometers and HR transmitter belts had to be removed for aquatic activities.

5.2.5 Data analysis:

Descriptive statistics included means, standard deviation or (range) following a Shapiro Wilk test for normality. In order to identify the relationship between total volume of physical activity (as evaluated by CPM (uniaxial and triaxial) and TEE, AEE and PAL, hierarchically nested regression models were used. The regression models for TEE, AEE and PAL included BM, CPM ActiTrainer, ΔHR_{acc} and CPM 3DNX (uniaxial and triaxial) as independent variables. Non linear models were also explored (i.e., square of body mass, square of CPM). Significance was set at $P<0.05$. All statistical analysis was completed using the software package SPSS, Version 17.0 (SPSS, inc., Chicago, IL) and R version 2.14.1 (R Development Core Team, 2011).

5.3 Results

Descriptive statistics of the child and adolescent subjects are presented in Table 5.1. Measured TEE and calculated AEE and PAL are presented in Table 5.1 for both children and adolescents. In children, none of the CPM ActiTrainer, CPM $3DNX_z$ or CPM $\sum 3DNX_{xyz}$ was significantly correlated with TEE ($P>0.05$). However, CPM ActiTrainer ($r = 0.4$, $P < 0.05$) and CPM $3DNX_z$ ($r = 0.3$, $P < 0.05$) were significantly correlated with AEE (Figure 5.1). Similarly, CPM ActiTrainer, CPM $3DNX_z$ and CPM $\sum 3DNX_{xyz}$ were significantly ($P<0.05$) correlated with PAL ($r = 0.47$, $r = 0.42$ and $r = 0.38$, for CPM ActiTrainer, CPM $3DNX_z$ and CPM $\sum 3DNX_{xyz}$ respectively) (Figure 5.3). On the other hand, in the adolescents, CPM ActiTrainer was not significantly correlated with TEE (Figure 5.2).

Table 5.1: Descriptive characteristics of the child and adolescent subjects

	Children		Adolescents	
	Mean ± SD	**[Range]**	**Mean ± SD**	**[Range]**
Subject (M/F)	24/25		15/15	
Age (yrs)	6.9 ± 1.5	[4 - 10]	14 ± 1	[10 - 17]
Weight (kg)	25 ± 7	[14 - 48]	41 ± 9	[25 - 57]
Height (cm)	121 ± 10	[101 - 140]	157 ± 11	[132 - 175]
BMI (kg/m^2)	16.6 ± 3.0	[13.1 - 26]	16 ± 2	[14 - 22]
BMI z score	0.45 ± 1.7	[-6.34 - 5.39]	-1.76 ± 3.96	[-3.29 - 0.45]
BMR (MJ/day)	4.27 ± 0.57	[3.23 - 6.04]	-	-
TEE (MJ/day)	6.6 ± 1.17	[3.94 - 10.3]	12.2 ± 3.5	[7 - 20.7]
AEE (MJ/day)	1.63 ± 0.6	[0.32 - 3.36]	-	-
PAL	1.5 ± 0.14	[1.2 - 1.8]	-	-
CPM (ActiTrainer)	566 ± 122	[310 - 857]	1165 ± 244	[811 - 1845]
CPM (3DNX)	816 ± 114	[562 - 1104]	-	-

† -Significantly different ($P<0.05$)
BMI - Body mass index
BMR - Basal metabolic rate
TEE - Total energy expenditure
AEE - Activity related energy expenditure
PAL - Physical activity level
CPM - Accelerometer counts per minute
- Not determined

TEE vs. CPM ActiTrainer: r = 0.23, P>0.05

†AEE vs. CPM ActiTrainer: r = 0.40, P<0.05

TEE vs. 3DNX$_z$: r = 0.12, P >0.05

†AEE vs. 3DNX$_z$: r = 0.3, P < 0.05

† - Significant association (P<0.05)

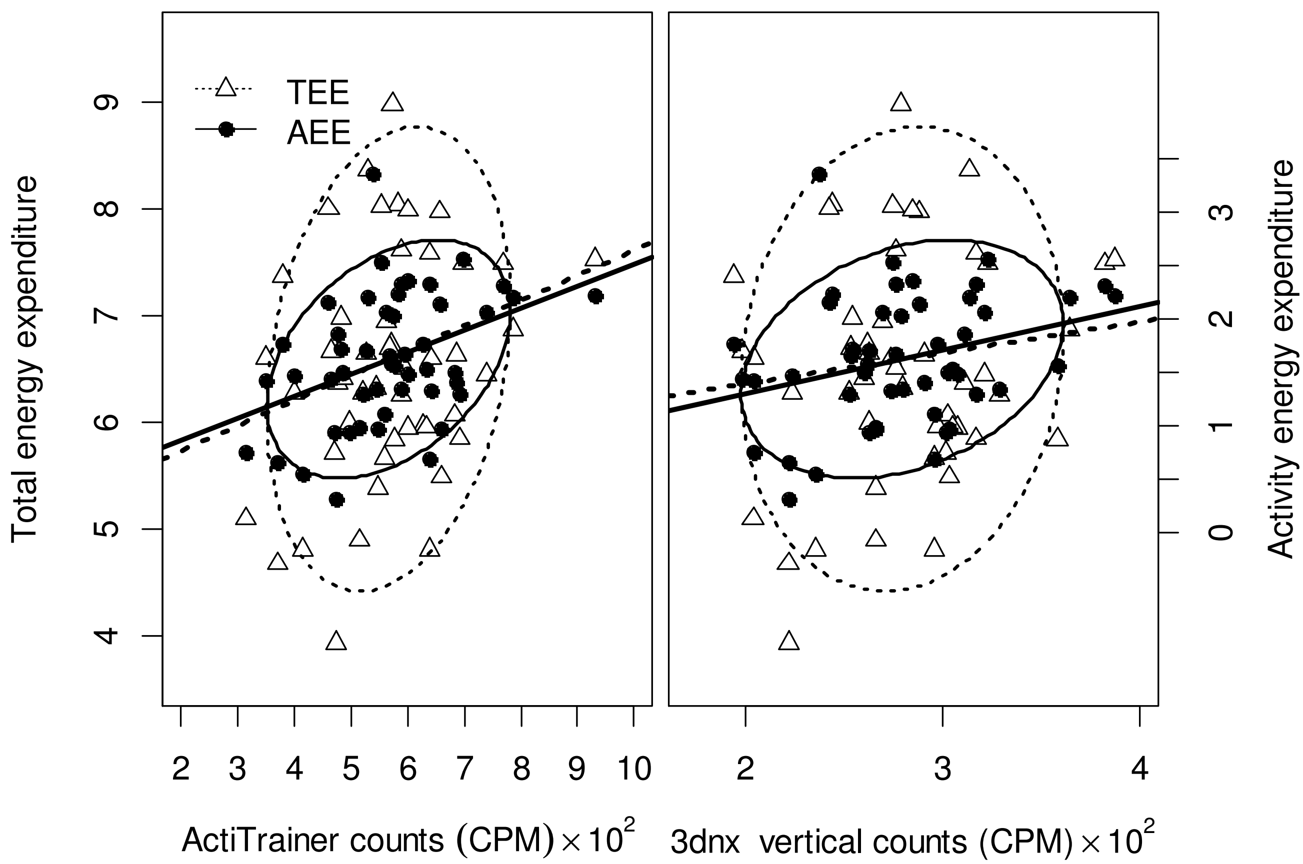


Figure 5.1: The relationship between ActiTrainer and 3DNX accelerometer outputs with total energy expenditure (TEE) and activity induced energy expenditure (AEE) (with linear regression lines and 80% prediction confidence ellipses) in children. Under bivariate normality, the percentage of observations falling inside the ellipse should closely agree with the specified confidence level. The confidence ellipse collapses diagonally as the correlation between two variables approaches 1 or -1. The confidence ellipse is more circular when two variables are uncorrelated.

TEE vs. CPM ActiTrainer: r = 0.23, P > 0.05

†AEE vs. CPM ActiTrainer: r = 0.40, P < 0.05

† - Significant association (P<0.05)

TEE vs. ∑3DNX: r = 0.02, P > 0.05

AEE vs. ∑3DNX$_z$: r = 0.21, P < 0.05

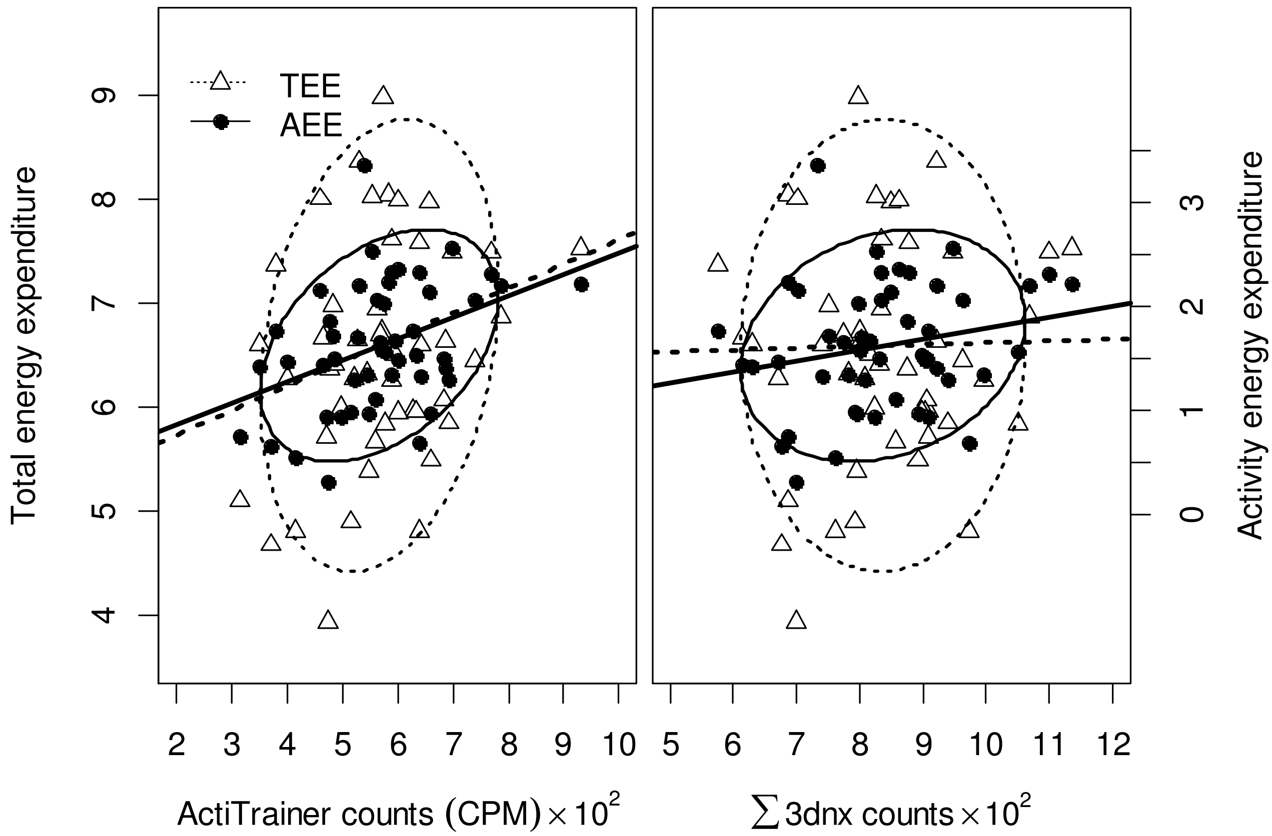


Figure 5.1: The relationship between ActiTrainer and 3DNX accelerometer outputs with total energy expenditure (TEE) and activity induced energy expenditure (AEE) (with linear regression lines and 80% prediction confidence ellipses) in children. Under bivariate normality, the percentage of observations falling inside the ellipse should closely agree with the specified confidence level. The confidence ellipse collapses diagonally as the correlation between two variables approaches 1 or -1. The confidence ellipse is more circular when two variables are uncorrelated.

TEE vs. CPM ActiTrainer: r = 0.15, P > 0.05

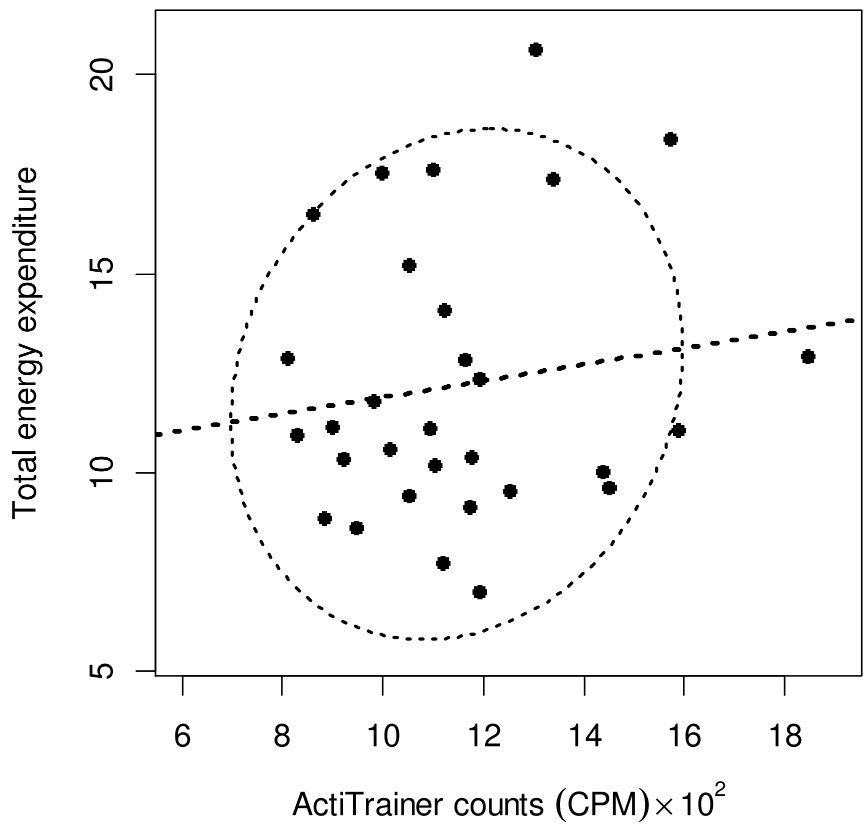


Figure 5.2: The relationship between ActiTrainer accelerometer outputs with total energy expenditure (TEE) (with linear regression lines and 80% prediction confidence ellipses) in adolescents. Under bivariate normality, the percentage of observations falling inside the ellipse should closely agree with the specified confidence level. The confidence ellipse collapses diagonally as the correlation between two variables approaches 1 or -1. The confidence ellipse is more circular when two variables are uncorrelated.

†PAL vs. CPM ActiTrainer: r = 0.47, P<0.05 PAL vs. $\sum 3DNX_z$: r = 0.42, P<0.05

† - Significant association (P<0.05)

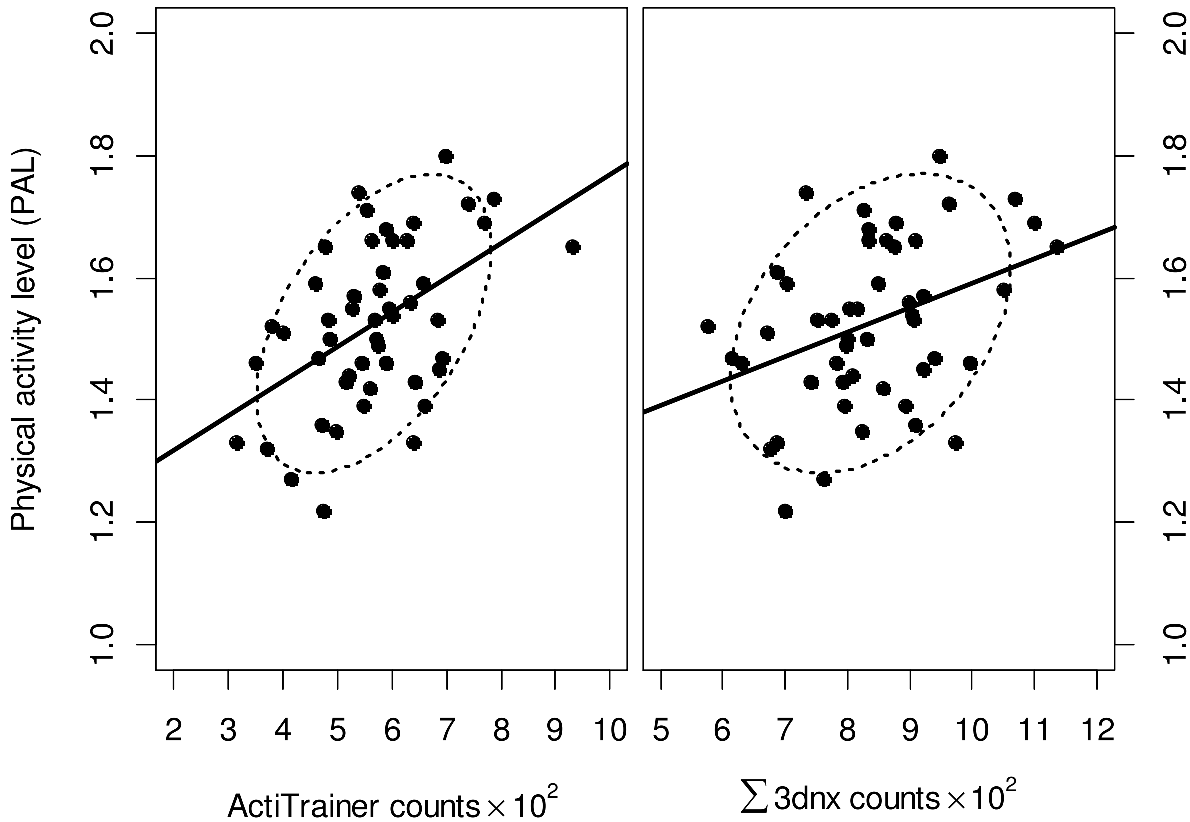


Figure 5.3: The relation between ActiTrainer and 3DNX accelerometer outputs with PAL (with linear regression lines and 80% data ellipses) in young children. Under bivariate normality, the percentage of observations falling inside the ellipse should closely agree with the specified confidence level. The confidence ellipse collapses diagonally as the correlation between two variables approaches 1 or -1. The confidence ellipse is more circular when two variables are uncorrelated.

5.3.1 TEE prediction models in children and adolescents using uniaxial and triaxial accelerometer outputs:

In children, 86% of the variance in TEE could be predicted by a model combining BM (Partial r^2 = 71%, P<0.05), CPM ActiTrainer (Partial r^2 = 11%, P<0.05) and ΔHR_{acc} (Partial r^2 = 4%, P<0.05) (Table 5.2). Similarly, 79% of the variance in TEE could be predicted by a model combining BM (Partial r^2 = 71%, P<0.05) and CPM $\sum 3DNX_{xyz}$ (Partial r^2 = 8%, P<0.05) (Table 5.2). However, a model examining the predictive validity of the individual 3DNX axes indicated that 81% of the variance in TEE could be predicted by BM (Partial r^2 = 71%, P<0.05) and $3DNX_z$ (Partial r^2 = 10%, P<0.05). Addition of $3DNX_x$ and $3DNX_y$ improved the model R^2 by 3% which was not significant (P>0.05) (Table 5.2). The SEE of TEE estimate for ActiTrainer and 3DNX models ranged from 0.44 - 0.74 MJ/d or approximately 6 - 11% of the average TEE (Table 5.2). Height, age and gender and other HR variables were not significant predictors of TEE in children. In adolescents on the other hand, 39% of the variance in TEE could be predicted by BM (Partial r^2 = 19%, P<0.05), CPM ActiTrainer (Partial r^2 = 15%, P<0.05) and ΔHR_{acc} (Partial r^2 = 5%, P<0.05) (Table 5.3). The S. E of TEE estimate for ActiTrainer models ranged from 3.2 - 5.5 MJ/d or approximately 26 - 45% of the average TEE (Table 5.3). Height, age and gender and other HR variables were not significant predictors of TEE in adolescents.

Table 5.2: Comparison of the accuracy of total energy expenditure (TEE) prediction models using body mass plus uniaxial accelerometry with heart rate vs. triaxial accelerometry in children

Model	R^2	Δr^2	SEE
TEE = 0.15[BM] + 2.76 MJ/d	71%		0.37
TEE = 0.16[BM] + 0.003[ActiTrainer] + 0.72 MJ/d	82%	11%	0.49
TEE = 0.15[BM] + 0.003[ActiTrainer] + 0.04[ΔHR_{acc}] + 0.38 MJ/d	86%	15%	0.44
TEE = 0.17[BM] + 0.009[$3DNX_z$] + 0.03 MJ/d	81%	10%	0.64
TEE = 0.16[BM] + 0.01[$3DNX_z$] - 0.003[$3DNX_x$] + 0.31 MJ/d	81%	10%	0.71
TEE = 0.16[BM] + 0.02[$3DNX_z$] - 0.004[$3DNX_x$] - 0.006[$3DNX_y$] + 0.61 MJ/d	83%	12%	0.71
TEE = 0.18[BM] + 0.003[$\sum 3DNX_{xyz}$] + 0.13 MJ/d	79%	8%	0.74

CPM ActiTrainer - Counts per min of uniaxial ActiTrainer accelerometer contribution to the regression coefficient
CPM $3DNX_z$ - Counts per min of Z-axis (longitudinal) contribution to the regression coefficient
CPM $3DNX_x$ - Counts per min of X-axis (anteroposterior) contribution to the regression coefficient
CPM $3DNX_y$ - Counts per min of Y-axis (mediolateral) contribution to the regression coefficient
CPM $\sum 3D\ NX_{xyz}$ - Counts per min of triaxial sum of 3DNX accelerometer contribution to the regression coefficient
ΔHR_{acc} - difference in HR during sedentary and moderate activities determined by accelerometry
R^2 - Regression coefficient
Δr^2 - change in R^2 relative to the first model containing body mass as the single predictor
TEE - Total energy expenditure
BM - Body mass
SEE - Standard error of estimate

Table 5.3: Comparison of the accuracy of total energy expenditure (TEE) prediction models using body mass plus uniaxial accelerometry with heart rate monitoring in adolescents

Model	R^2	Δr^2	SEE
TEE = 0.19[BM] + 4.45 MJ/d	19%		3.2
TEE = 0.26[BM] + 0.006[ActiTrainer] - 5.34 MJ/d	34%	15%	5.2
TEE = 0.26[BM] + 0.006[ActiTrainer] - 0.14[ΔHR_{acc}] - 2.36 MJ/d	39%	20%	5.5

CPM ActiTrainer - Counts per min of uniaxial ActiTrainer accelerometer contribution to the regression coefficient
ΔHR_{acc} - difference in HR during sedentary and moderate activities as determined by accelerometry
R^2 - Regression coefficient
Δr^2 - change in R^2 relative to the first model containing body mass as the single predictor
TEE - Total energy expenditure
BM - Body mass
SEE - Standard error of estimate

5.3.2 AEE prediction models in children using uniaxial and triaxial accelerometer outputs:

61% of the variation in AEE could be explained by BM (Partial r^2 = 35%, P<0.05), CPM ActiTrainer (Partial r^2 = 20%, P<0.05) and ΔHR_{acc} (Partial r^2 = 4%: P<0.05) (Table 5.4). Similarly, 51% of the variance in AEE could be predicted by BM (Partial r^2 = 35%, P<0.05) and CPM $\sum 3DNX_{xyz}$ (Partial r^2 = 16%, P<0.05) (Table 5.4). However, a model examining the predictive validity of individual 3DNX axes indicated that 55% of the variance in AEE could be predicted by BM (Partial r^2 = 35%, P<0.05), and $3DNX_z$ (Partial r^2 = 20%, P<0.05). Addition of $3DNX_x$ and $3DNX_y$ improved the model R^2 by 3% but this contribution was not significant (P>0.05). The S.E of AEE estimates ranged from 0.28 - 0.57 MJ/d or 17 - 35% of the average AEE for the CPM ActiTrainer and CPM $\sum 3DNX_{xzy}$ respectively (Table 5.4). Height, age and gender were not significant predictors of AEE in children.

Table 5.4: Comparison of the accuracy of activity induced energy expenditure (AEE) prediction models using body mass plus uniaxial accelerometry with heart rate vs. triaxial accelerometry in children.

Model	**R^2**	**Δr^2**	**SEE**
AEE = 0.06[BM] + 0.25 MJ/d	35%		0.28
AEE = 0.06[BM] + 0.002[ActiTrainer] - 1.24 MJ/d	57%	22%	0.40
AEE = 0.06[BM] + 0.002[ActiTrainer] + 0.02[ΔHR_{acc}] - 1.42 MJ/d	61%	26%	0.38
AEE = 0.07[BM] + 0.006[$3DNX_z$] - 1.76 MJ/d	55%	20%	0.50
AEE = 0.06[BM] + 0.008[$3DNX_z$] - 0.002[$3DNX_x$] - 1.6 MJ/d	56%	21%	0.56
AEE = 0.06[BM] + 0.01[$3DNX_z$] - 0.002[$3DNX_x$] - 0.003[$3DNX_y$] -1.42 MJ/d	58%	22%	0.57
AEE = 0.07[BM] + 0.002[$\sum 3DNX_{xyz}$] - 1.76 MJ/d	51%	16%	0.56

CPM ActiTrainer - Counts per min of uniaxial ActiTrainer accelerometer contribution to the regression coefficient
CPM $3DNX_z$ - Counts per min of Z-axis (longitudinal) contribution to the regression coefficient
CPM $3DNX_x$ - Counts per min of X-axis (anteroposterior) contribution to the regression coefficient
CPM $3DNX_y$ - Counts per min of Y-axis (mediolateral) contribution to the regression coefficient
CPM $\sum 3DNX_{xyz}$ - Counts per min of triaxial sum of 3DNX accelerometer contribution to the regression coefficient
ΔHR_{acc} - difference in HR during sedentary and moderate activities as determined by accelerometry
R^2 - Regression coefficient
Δr^2 - change in R^2 relative to the first model containing body mass as the single predictor
AEE - Activity induced energy expenditure
BM - Body mass
SEE - Standard error of estimate

Table 5.5: Comparison of the accuracy of physical activity level (PAL) prediction models using body mass plus uniaxial accelerometry with heart rate vs. triaxial accelerometry in children.

Model	R^2	Δr^2	SEE
PAL = 0.006[BM] + 1.38	8%		0.07
PAL = 0.007[BM] + 0.001[ActiTrainer] + 1	37%	29%	0.1
PAL = 0.006[BM] + 0.001[ActiTrainer] + 0.004[ΔHR_{acc}]	40%	32%	0.1
PAL = 0.009[BM] +0.002[$3DNX_z$] + 0.86	37%	29%	0.1
PAL = 0.008[BM] + 0.002[$3DNX_z$] - 0.0004[$3DNX_x$] + 0.9	37%	29%	0.15
PAL = 0.008[BM] + 0.003[$3DNX_z$] - 0.001[$3DNX_x$] - 0.001[$3DNX_y$] + 0.94	40%	32%	0.15
PAL = 0.009[BM] + 0.001[$\sum 3DNX_{xyz}$] + 0.86	31%	23%	0.15

CPM ActiTrainer - Counts per min of uniaxial ActiTrainer accelerometer contribution to the regression coefficient
CPM $3DNX_z$ - Counts per min of Z-axis (longitudinal) contribution to the regression coefficient
CPM $3DNX_x$ - Counts per min of X-axis (anteroposterior) contribution to the regression coefficient
CPM $3DNX_y$ - Counts per min of Y-axis (mediolateral) contribution to the regression coefficient
CPM $\sum 3DNX_{xyz}$ - Counts per min of triaxial sum of 3DNX accelerometer contribution to the regression coefficient
ΔHR_{acc} - difference in HR during sedentary and moderate activities as determined by accelerometry
R^2 - Regression coefficient
Δr^2 - change in R^2 relative to the first model containing body mass as the single predictor
PAL - Physical activity level BM - Body mass SEE - Standard error of estimate

5.3.3 PAL prediction models in children using uniaxial and triaxial accelerometer outputs:

40% of the variation in PAL could be explained by BM (Partial r^2 = 8%, $P<0.05$), CPM ActiTrainer (Partial r^2 = 29%, $P<0.05$) and ΔHR_{acc} (Partial r^2 = 3%: $P<0.05$) (Table 5.5). Similarly, 31% of the variance in PAL could be predicted by BM (Partial r^2 = 8%, $P<0.05$) and CPM $\sum 3DNX_{xyz}$ (Partial r^2 = 23%, $P<0.05$) (Table 5.5). However, a model examining the predictive validity of individual 3DNX axes indicated that 37% of the variance in PAL could be predicted by BM (Partial r^2 = 8%, $P<0.05$), and $3DNX_z$ (Partial r^2 = 29%, $P<0.05$). Addition of $3DNX_x$ and $3DNX_y$ improved the model R^2 by 3% but this contribution was not significant ($P>0.05$). The S.E of PAL estimates ranged from 0.07 - 0.15 or 5-10% of the average PAL for the CPM ActiTrainer and CPM $\sum 3DNX_{xyz}$ respectively (Table 5.5). Height, age and gender were not significant predictors of PAL in the children.

5.4 Discussion:

Energy expenditure can be estimated by measuring body acceleration (Yang & Hsu, 2010). The DLW and indirect calorimetry are regarded as the gold standard references of energy expenditure assessment (Yang & Hsu, 2010). Though accurate, gas analyzers for indirect calorimetry are expensive and they require specialized skills to operate (Yang & Hsu, 2010). Therefore, accelerometers provide an alternative method of estimating energy expenditure in a free living environment (Yang & Hsu, 2010). In children, ActiTrainer with HR monitoring and 3DNX reported comparable model prediction accuracy for free living TEE and AEE with similar models obtained with the $Tracmor_D$ accelerometer in adults (Bonomi *et al*., 2010) based on reported SEE and partial r^2 values. In addition, there was significant positive association between both ActiTrainer and 3DNX outputs and PAL (Figure 5.3) which contradict the findings of Carter *et al*., (2008) and is probably due to the fact that they used an earlier generation of the 3DNX accelerometer, lower subject numbers i.e. 37 compared to 49 in the current study or sample specific differences between the two studies. Furthermore, uniaxial ActiTrainer and $3DNX_z$ reported comparable validity relative to $\sum 3DNX_{xyz}$ accelerometer and $3DNX_x + 3DNX_y + 3DNX_z$. For the triaxial 3DNX accelerometer therefore, addition of the anteroposterior and mediolateral axes to the longitudinal axis did not significantly contribute to the energy expenditure model prediction in children. In adolescents on the other hand, a model combining BM with actiTrainer outputs predicted 34% of the variance in TEE compared to 82% in children. However, the relative contribution of the ActiTrainer outputs to the model prediction was 11% and 15% for children and adolescent subjects respectively. Thus, BM was the principal determinant of TEE in children. In adolescents however, BM and ActiTrainer count were equally important determinants of TEE, which may reflect the fact that accelerometry may have better utility in assessing energy expenditure in the more active adolescent sample compared to the relatively inactive child sample. In both cohorts, addition of ΔHR_{acc} monitoring to ActiTrainer outputs in the energy expenditure prediction models improved the prediction accuracy by 3 - 5%. This is consistent with the observation that addition of HR to accelerometry improves energy expenditure prediction (Heskell, 1993).

However, the relationship between HR and instantaneous AEE is approximately linear during moderate to vigorous activity intensities but not at low intensity activity and while sedentary (Livingtone et *al.,* 1992), Therefore, to avoid the problems associated with use of HR at low physical activity intensities to predict energy expenditure it has been suggested that HR monitoring should only be used to assess time spent in moderate and vigorous intensity activity in children (Riddoch *et al.,* 1995). This may explain why use of ΔHR_{acc}- a variant of HR reserve (Corder et al., 2008), defined in this thesis as the difference between moderate HR and sedentary HR, and which incorporates some level of individual calibration by the using sedentary HR was significantly associated with energy expenditure.

The ActiTrainer ActiGraph accelerometer outputs were a significant predictor of energy expenditure in children and adolescents. Furthermore, addition of HR monitoring improved the predictive validity of the ActiTrainer. This contradicts the finding that activity measured using ActiGraph accelerometers is not related to AEE and PAL and thus ActiGraph accelerometers may not be useful in predicting AEE in children (Krishnaveni *et al*., 2009) but is supported by the findings of Ekelund *et al*., (2001). Examination of the validity of the models combining accelerometer outputs (CPM) and anthropometric measures to predict TEE, AEE and PAL consistently indicated that uniaxial ActiTrainer outputs combined with HR monitoring were comparable to triaxial 3DNX outputs in children. However, comparison of these findings with previous prediction models is difficult because of differences in the activity profiles between study groups, different independent variables included in the regression such as Fat Free Mass and Sleeping metabolic rate, different age groups (Bonomi *et al*., 2010), different devices used in other studies and other sample specific differences. This notwithstanding, use of accelerometer outputs to predict energy expenditure shows significant promise if accelerometer prediction accuracy of energy expenditure is improved by addressing specific issues related to accelerometer based measurements of physical activity, such as intraindividual variability in AEE (Bonomi *et al*., 2009), interindividual variability in accelerometer outputs in subjects performing standardized activities (Welk, 2005) and lack of comparability between accelerometer counts from different manufacturers (Chen & Bassett, 2005). However, recently van Hees and colleagues (2011) used a GENEA (Unilever Discover, UK) accelerometer that has capability to record raw accelerometer data in *g* units and therefore this device may be practical in attempts to standardize accelerometer outputs. Most devices however, such as the ActiTrainer and 3DNX used in this study do not have sufficient memory to store several days worth of raw data in *g* units. Furtheremore, the raw data are already processed into counts using proprietary algorithims and technically there is no way of converting counts back to the original *g* units, hence inter device comparison of counts may not be feasible.

According to Trost *et al*. (2005) evidence indicates that some accelerometers may perform better than others under certain conditions, but the reported differences are not consistent or sufficiently compelling to single out one brand or type of accelerometer as being superior to the others. When it comes to selecting an accelerometer, issues of affordability, product reliability, monitor size, technical support, and comparability with other studies may be equally as important as the relative validity and reliability of an instrument. It is necessary to begin systematically evaluating the absolute and concurrent validity of these instruments under a variety of conditions such as in children and adolescent cohorts used in this study. This can be accomplished only by comparing multiple instruments under the same conditions and against a suitable ‘gold standard’ (Welk *et al*., 2000) since the relative validity and interinstrument reliability of a given accelerometry product is of primary importance. Furthermore, accelerometer validation studies should report both r^2 and SEE values for comparison of device validity.

Uniaxial accelerometry with HR monitoring and triaxial accelerometry were comparable in the assessment of free living energy expenditure in young children. This is consistent with previous studies (Welk *et al*., 2000, Puyau *et al*., 2002) but contradicts the findings of Plasqui *et al*. (2005) in adults who reported that to measure the wide variety of daily life activities triaxial accelerometers are more suitable than uniaxial. Intuitively, it is expected that the sum of body accelerations in the three axes would be a better predictor of energy expenditure associated with motion. Therefore, triaxial accelerometers in general clearly provide more information which could provide marginal improvements in validity in other samples (e.g., older children, more active, more diverse/complex activities besides occasional walking). However, this was not the case for the 3DNX accelerometer in the child cohort, since only the longitudinal axis was a significant predictor of energy expenditure.

To conclude, uniaxial ActiTrainer with HR monitoring and triaxial 3DNX accelerometry have comparable validity in assessing free living energy expenditure in young children.

Furthermore, ActiTrainer outputs were valid for assessing energy expenditure in active adolescents. However, there is a wide array of activity monitors which have yet to be properly validated and therefore accuracy of most remains to be determined. In addition, there is a need to explore further the relationship between energy expenditure and physical activity intensity/patterns rather than just physical activity volume (as assessed by CPM).

Chapter 6

6.0: The relation between free living physical activity, energy expenditure and indices of adiposity in children and adolescents

6.1 Introduction:

Western societies have experienced an obesity epidemic over the past 40 years (Wang & Lobstein, 2006, Flegal *et al*., 2010, Onis *et al*., 2010) with over 25% of the population of many such societies currently classed as obese (body mass index BMI >30) and an additional 40 - 45% overweight (BMI >25) (Wang & Lobstein, 2006, Flegal *et al*., 2010, Onis *et al*., 2010). Obesity is a problem caused by prolonged energy imbalance where energy intake exceeds energy expenditure (Tappy *et al*., 2003). Whether this has been a consequence of elevated food consumption, or reduced physical activity remains a matter of debate. Over the time course of the epidemic there have been large changes in our physical activity patterns. Sedentary leisure time activities such as TV viewing and computer use have increased enormously relative to the 1950s (Dollman *et al*., 2005). However, whether these trends in activity have been sufficient to impact on energy expenditure is uncertain. There has apparently been no reduction in the level of energy expenditure between the 1980s and the present day in adults in western societies (Westerterp & Speakman, 2008), and estimates of the gap between intake and expenditure necessary to drive the epidemic suggest that this can be entirely accounted for by differences in food consumption rather than expenditure (Swinburn *et al*., 2009, Hall, 2010). Moreover, measurements of energy demands in rural communities that are at a lesser stage of economic development that may precede a potential epidemic indicate no significant difference to those of western societies (Westerterp & Speakman, 2008, Luke *et al*., 2009), despite the suggested higher levels of physical activity of individuals in such communities.

One reason why expenditures in rural communities may not be significantly higher than in western societies is that our impressions of the levels of expenditure may be overly influenced by observations of occasional periods of vigorous activity. Such periods, however, may be compensated for by increases in the time spent in low levels of activity at other times of day. In addition, although we have certainly increased the time we spend watching TV, this shift may have occurred in a time slot in the early evening where it may have only displaced other sedentary pursuits such as reading or listening to the radio. On the other hand, a recent systematic review on the trends of energy expenditure in children and adolescents over time indicated that absolute TEE had declined, but this was only evident after adjusting for the increasing body mass which had occurred over the period of the review (Malina & Little, 2008). In addition, another review on the trends of physical activity over the past 50 years in US adults indicated a decline in occupational related energy expenditure which would account for increased body weights in men and women (Church *et al*., 2011).

Previous studies on Kenyan adolescents living in rural areas indicated a highly physically active lifestyle, with subjects reporting that they walked or ran on average for 3 h/d and also spent an average of 40 min/d working in the fields (Larsen *et al*., 2004, Onywera *et al*., 2006). This high level of reported activity would be expected to lead to high levels of TEE compared to levels observed in European children living in industrialised countries.

The aim of the current study was therefore, to determine the energy demands of a rural population of Kenyan adolescents and young urbanised European children using the DLW technique at the same time as determining the relationship between adiposity, physical activity levels and patterns and energy expenditure in both cohorts.

6.2 Material and methods:

6.2.1 Subjects:

A subsample of 60 children (32 female, 28 male) from the initial child cohort fulfilled the inclusion criteria for analysis i.e. at least 6 weekdays including at least 1 weekend day of valid recording of at least 360 min of continuous monitoring/d as determined in Chapter 3. Additionally, a subsample of 30 rural Kenyan adolescents (15 male, 15 female), mean age 14 ± 1 y from the initial adolescent cohort were recruited to participate in this study (see section 2.1.3 for details). All subjects fulfilled the inclusion criteria for data analysis i.e. at least 4 weekdays of monitoring, including at least 1 weekend day of valid recording of at least 360 min of continuous monitoring/d as determined in Chapter 3.

6.2.2 Calorimetry:

TEE was measured over 7 day under free living conditions using the DLW technique in children and adolescents as outlined in section 5.2.2

6.2.3 Accelerometry:

Free living daily physical activity levels and patterns were objectively quantified using the ActiTrainer uniaxial accelerometers in both cohorts as outlined in section 5.2.3. In addition, for the adolescent sample, subjects were simultaneously fitted with either a Timex trainer V 1.3.36 (Timex Group, USA) or GPSports Team AMS Release 1.2.1.12 (Fyshwick, Australia) GPS devices during their travel from home to school to obtain daily distance travelled to school. Physical activity was expressed as total volume of physical activity (average CPM) over the monitoring period. To convert accelerometry output to estimates of sedentary time and activities of different physical activity intensities, the recently validated Evenson cutpoints (2008) for children and adolescents using ActiGraph accelerometers were used. These are: sedentary, ≤100 CPM, light, >100 and <2296 CPM, moderate, >2296 and <4012 CPM, and vigorous physical activity, ≥4012 CPM. In addition, moderate to vigorous activity (MVPA) was calculated by combining moderate and vigorous categories (i.e. >2296 CPM).

6.2.4 Statistical analysis:

Descriptive statistics included calculations of means, standard deviation and range following a Shapiro Wilk test of normality. Time spent sedentary and in vigorous activities was not normally distributed. Differences between child and adolescent samples in physical characteristics and energy expenditure measurements were tested by General Linear Modelling. To identify the factors that may be associated with TEE and objectively measured physical activity and sedentary time in the present study, total amount of physical activity (as evaluated by CPM), sedentary time and physical activity intensity (time in light, moderate, vigorous and MVPA) were assessed by hierarchical nested regression analysis. The regression models for TEE included BM and physical activity parameters as independent variables. In addition, the relationship between indices of adiposity (i.e. BMI and BMI z score) and TEE or distance to school (active commuting) was examined by regression analysis. Statistical computations were performed using the software package SPSS, Version 17.0 (SPSS, inc., Chicago, IL).

6.3 Results:

Descriptive characteristics including anthropometrics and TEE values for both children and adolescents are summarized in Table 6.1. Adolescent subjects spent 49% of the monitored time engaged in light, moderate and vigorous activities compared to 45% in children. Similarly, adolescent subjects engaged in significantly ($P<0.05$) higher volumes of physical activity compared to children: 1148 ± 244 vs. 567 ± 118 CPM, for adolescent and child cohorts, respectively. Similarly, adolescent subjects engaged in significantly ($P<0.05$) more moderate, vigorous and MVPA compared to children (Table 6.2). Furthermore, adolescents travelled for and average distance of 7.5 ± 3 to school daily (Table 6.2).

6.3.1: Relationship between physical activity, energy expenditure and indices of adiposity:

In children, 71% of the variance in TEE could be predicted by BM ($P<0.05$). A model combining BM and CPM predicted 82% of the variance in TEE ($P<0.05$). In addition, a model combining BM, CPM and time spent engaged in MVPA predicted 85% of the variance in TEE ($P<0.05$) (Table 6.3). The S. E of TEE estimate ranged from 0.37 - 049 MJ/d or 6 - 7% of the average TEE. In adolescents on the other hand, 22% of the variance in TEE could be predicted by BM ($P<0.05$). A model combining BM and CPM predicted 34% of the variance in TEE ($P<0.05$). In addition, a model combining BM, CPM and time engaged in light activities predicted 42% of the variance in TEE ($P<0.05$) (Table 6.4). The standard error (S.E.) of TEE estimates for the models ranged from 2.8 – 6.4 MJ/d or 21 – 50% of the average TEE (Table 6.4). Furthermore, in adolescents, there was no association between variation in distance travelled to school (active commuting) and TEE. On the other hand, there was moderate negative correlation between BMI z scores and CPM ($r = -0.6$, $P = 0.001$), vigorous activities ($r = -0.53$, $P = 0.03$) and MVPA ($r = -0.57$, $P = 0.01$). In contrast, there was no association between BMI z scores and active commuting ($P >0.05$). Similarly, in children, there was no association between physical activity and BMI z scores. However, there was a weak association between time engaged in sedentary activities and BMI z scores ($r = 0.27$, $P<0.05$).

Table 6.1: Descriptive characteristics of the child and adolescent subjects

	Children		Adolescents	
	Mean ± SD	**[Range]**	**Mean ± SD**	**[Range]**
Subject (M/F)	28/32		15/15	
Age (yrs)	6.9 ± 1.5	[4 - 10]	14 ± 1†	[10 - 17]
Weight (kg)	25 ± 7	[14 - 48]	41 ± 9†	[25 - 57]
Height (cm)	121 ± 10	[101 - 140]	157 ± 11†	[132 - 175]
BMI (kg/m^2)	16.6 ± 3.0	[13.1 - 26]	16 ± 2	[14 - 22]
BMI z score	0.45 ± 1.7	[-6.34 - 5.39]	-1.76 ± 3.96	[-3.29 - 0.45]
TEE (MJ/day)	6.6 ± 1.2	[3.9 - 10.3]	12.2 ± 3.5†	[7 - 20.7]

† -Significantly different (P<0.05)
- Not determined
BMI - Body mass index
BMR - Basal metabolic rate
TEE - Total energy expenditure
AEE - Activity induced energy expenditure
PAL - Physical activity level
CPM - Accelerometer counts per minute

Table 6.2: Physical activity parameters in children and adolescents

	Children		Adolescents	
	Mean ± SD	**[Range]**	**Mean ± SD**	**[Range]**
CPM	567 ± 118	[313 - 931]	1148 ± 244†	[809 - 1843]
Sedentary (min)	394 ± 53	[298 - 502]	406 ± 63	[328 - 561]
Light (min)	275 ± 46	[160 - 402]	244 ± 56	[132 - 354]
Moderate (min)	40 ± 12	[16 - 69]	75 ± 18†	[43 - 113]
Vigorous (min)	15 ± 7	[1 - 32]	82 ± 30†	[32 - 153]
MVPA (min)	55 ± 18	[19 - 98]	156 ± 33†	[109 - 234]
% time sedentary	54	[40 - 73]	50	[39 - 67]
% time light	38	[24 - 52]	30	[17 - 42]
% time moderate	5	[2 - 9]	9	[5 - 17]
% time vigorous	2	[0 - 5]	10	[4 - 19]
% time MVPA	8	[3 - 13]	19	[14 - 30]
Distance to school (km)	-	-	7.5 ± 3	[0.8 - 13.4]

CPM - Counts per minute
MVPA - Moderate to Vigorous Physical activity
† - significantly different ($P<0.05$)

Table 6.3: Total energy expenditure (TEE) prediction models using body mass plus physical activity levels and patterns in children

Model	**R^2**	**Δr^2**	**SEE**
TEE = 0.15[BM] - 2.76 MJ/d	71%		0.37
TEE = 0.16[BM] + 0.003[CPM] + 0.72 MJ/d	82%	11%	0.49
TEE = 0.15[BM] + 0.85[MVPA] + 5.29 MJ/d	74%	3%	0.39
TEE = 0.15[BM] + 0.003[CPM] + 0.85[MVPA] + 5.28 MJ/d	85%	14%	0.47

BM - Body mass
CPM - Average accelerometer counts per minute
MVPA - Time spent engaged in moderate to vigorous activities
SEE - Standard Error of Estimate
TEE - Total energy expenditure
R^2 - Regression coefficient
Δr^2 - change in R^2 relative to the first model containing body mass as the single predictor

Table 6.4: Total energy expenditure (TEE) prediction models using body mass plus physical activity levels and patterns in adolescents

Model	**R^2**	**Δr^2**	**SEE**
TEE = 0.19[BM] + 4.45 MJ/d	22%		2.8
TEE = 0.25[BM] + 0.005[CPM] - 4.2 MJ/d	34%	12%	4.6
TEE = 0.24[BM] +16.8[Light] - 2.6 MJ/d	30%	8%	4.9
TEE = 0.32[BM] + 0.006[CPM] + 21.1[Light] - 14.5 MJ/d	42%	20%	6.1

BM – Body mass
CPM – Average accelerometer counts per minute
Light – Time spent engaged in Light activities
SEE – Standard Error of Estimate
TEE - Total energy expenditure
R^2 - Regression coefficient
Δr^2 – change in R^2 relative to the first model containing body mass as the single predictor

6.4 Discussion:

This study objectively assessed sedentary behaviour, physical activity volumes and patterns in relation to TEE and its components in a sample of European children and Kenyan adolescents. The results from the present study indicate that adolescents had higher engagement in physical activity compared to children. Habitual physical activity in Kenyan adolescents was very high compared to levels previously reported in Western children using the Evenson cutpoints (Kwon *et al*., 2011). Examination of the activity time budget indicated that Kenyan adolescents spent approximately 49% of the monitored time engaged in light, moderate and vigorous intensity physical activities compared to 45% in children. However, engagement in vigorous activity was 2% and 9% of the monitored time for the child and adolescent samples respectively. Thus, engagement in vigorous intensity physical activity contributes minimally to the activity time budget in both cohorts. This finding is consistent with previous findings in other populations in children and adults (Westerterp, 2001, Montgomery *et al*., 2004). Although adolescents spent relatively little time in vigorous intensity physical activity (75 min per day on average) this was still substantially higher than levels observed in European children or those previously reported in a free living American cohort using the Evenson cutpoints (Kwon *et al*., 2011).

All adolescents were lean, this notwithstanding, their absolute TEE was comparable to levels observed in obese European adolescents, primarily because of the high BMR observed in these obese subjects (Ekelund *et al*., 2002). Furthermore, Kenyan adolescents had a mean TEE of 12.2 MJ/d, which was comparable to the published average value of 11.2 MJ/d for adults in affluent societies (Black *et al*. 1996) in spite of the larger BM and hence BMR of adult subjects. It may therefore be inferred that AEE is a highly significant component of TEE in lean Kenyan adolescents. Therefore, the role of reduced energy expenditure in fuelling the obesity epidemic needs to be examined more closely.

Recent evidence indicates that levels of habitual physical activity of children and adolescents earlier in the century (preindustrial) were greater compared with contemporary youth in postindustrial societies (Malina & Little, 2008) which is consistent with the study findings comparing the postindustrial child cohort vs. the preindustrial adolescent cohort. Physical inactivity is implicated in the recent worldwide obesity epidemic (WHO, 2000). In fact, it has been previously reported that active commuting (a proxy of physical activity) is inversely related to obesity and has been suggested to explain the differences in global obesity prevalence rates (Bassett *et al*., 2008). However, there was no association between active commuting and either energy expenditure or indices of adiposity in the adolescent sample. Conversely, indices of physical activity (i.e. CPM and time in vigorous and MVPA) were negatively correlated with indices of adiposity in adolescent but not in the child sample.

In traditional poor societies whose economy is based on human labour, basic needs such as food are satisfied through investment in occupational physical activity (Bénéfice *et al*., 2001). Furthermore, active commuting to school is the principal mode of transport in rural Kenyan school children (Larsen *et al*., 2004, Onywera *et al*., 2006). Therefore, it was anticipated that variations in TEE would be largely the result of variations in physical activity. However, volume of physical activity (CPM) and time spent engaged in light activities were the only significant predictors of TEE. On the other hand, time engaged in moderate, vigorous and MVPA were not significant predictors of TEE. Conversely, in the child sample, which was a relatively sedentary cohort compared to adolescents in this study, CPM and time engaged in MVPA were significant predictors of TEE. It was anticipated that higher intensity physical activities would be more strongly associated with energy expenditure in active adolescents compared to inactive children based on the findings that adolescents engaged in significantly more high intensity physical activities compared to children. However, the *post hoc* statistical power calculations indicated that we needed a sample size of at least 37 (see section 2.1.4) but due to practical limitations we could only managed to sample 30 subjects, coupled with the wide variability in physical activity and energy expenditure parameters in the adolescent sample, significant associations may have been masked between physical activity patterns and energy expenditure. Therefore, associations between physical activity patterns and energy expenditure should be explored further in a much larger sample of free living active populations.

In conclusion, objectively assessed physical activity indicates that Kenyan adolescents are very active and have high TEE values compared to levels observed in European children and adolescents which may be a likely reflection of the active lifestyles these subjects engage in. Furthermore, physical activity was a significant predictor of TEE in both children and adolescents. However, there was no association between active commuting and TEE or indices of adiposity in Kenyan adolescents.

Chapter 7

7.0: The relation between the environment, physical activity and indices of adiposity in children and adolescents

7.1 Introduction

In a recent review it was estimated that by 2010 the European Union can expect the numbers of overweight and obese children to rise by approximately 1.3 million children/y, of which over 0.3 million/y will be obese (Jackson-Leach & Lobstein, 2006). In an attempt to counter this obesity epidemic in European children, IDEFICS study aims to enhance the knowledge of the impact of lifestyle related factors, such as physical activity, on children's health (Bammann *et al*., 2006). It is therefore essential to classify the current physical activity profiles of children in order to develop effective intervention programs that will enhance physical activity and reduce the risk of childhood obesity (Reilly, 2008).

Physical activity has a range of health related benefits for children and adolescents (Strong *et al*., 2005, USDHHS, 2008, Warbuton *et al.,* 2011) with recommendations that at least 60 min of MVPA should be accumulated daily (USDHHS, 2008, CMO 2011). Guidelines also exist which recommend that children and adolescents restrict the amount of time they spend in sedentary behaviour, a separate construct from physical activity

(AAP, 2007, CMO, 2011). Increased childhood sedentary behaviour has been proposed as a likely explanation for the increased secular trend in childhood obesity in the West (Rolland-Cachera *et al*., 1984, Bammann *et al*., 2006, Reilly, 2008).

Similarly, Sub Saharan Africa is known to be undergoing the 'epidemiological transition' as demonstrated by a reduction in childhood mortality due to communicable diseases and under nutrition (Omran, 2005). These changes may be explained by rapid urbanisation and adoption of Westernised lifestyles (Popkin, 2001). Urbanisation is often associated with extreme changes in dietary habits and habitual physical activity (Yamauchi *et al*., 2001), which may explain the observed differences in prevalence of lifestyle disorders such as obesity, hypertension and diabetes between rural and urban populations in Africa (Sobngwi *et al*., 2002). Rapid urbanisation has occurred in Kenya in recent years (Otiso & Owusu, 2008) but the extent to which urbanisation has affected habitual physical activity, sedentary behaviour and indices of adiposity in Kenyan adolescents is unknown.

There is mounting evidence that physical activity in particularly defined contexts, such as active transport, school physical education, and organised sport is waning in urbanised children in the West (Dollman & Norton, 2005). However, it remains to be determined whether these trends are replicated in urban areas in Africa, since it has been previously reported that Kenyan and Ethiopian children from rural areas run up to 20 km to school daily, and are also engaged in physically active leisure time activities and household chores (Scott *et al*., 2003, Larsen *et al*., 2004, Onywera *et al*., 2006). However, objective assessment of habitual physical activity levels using accelerometry in these populations remains to be determined. The primary purpose of the present study, therefore, was to compare habitual physical activity levels in children from urban areas and adolescents from rural and urban areas (environmental influence) using objective measures (i.e. accelerometry). A secondary aim was to test for relationships between objectively measured physical activity, sedentary behaviour and adiposity in children and adolescents in light of recent recommendations that at least 60 min of MVPA should be accumulated daily to maintain healthy weight (USDHHS, 2008, CMO, 2011).

7.2 Material and methods

7.2.1 Subjects:

A subsample of 60 children (32 female, 28 male) from the initial child cohort and a sub sample of 200 apparently healthy adolescents aged 12 - 16 y from the initial adolescent cohort fulfilled the inclusion criteria as determined in Chapter 3. Subjects from the child sample were from urbanised areas in the UK, Spain and Belgium (Figure 2.1). For the adolescent sample, the participants from rural areas were 97 (50 female and 47 male) and from urban areas 103 (52 female and 51 male) (see Table 7.1 and 7.2 for physical characteristics). Adolescent subjects were recruited from Eldoret Town Council (urban group) and Wareng County Council (rural group). Adolescent subjects were assigned to the urban group if they lived within the municipal boundaries of Eldoret town and were served at home with mains electricity and piped water. Eldoret is a town (2100-2700 m above sea level) in western Kenya and the administrative centre of Uasin Gishu District of Rift Valley Province with a population of 193,830 in 1999 (census) (GOK, 1999). In contrast, subjects were assigned to the rural group if the subjects lived in small and remote villages in the Nandi region of Kenya without access to mains electricity and piped water at home (Figure 2.1). Habitual physical activity levels were assessed by uniaxial ActiTrainer using Evenson cutpoints (2008) as described previously in Chapter 6. A physical activity diary was also used in the adolescent cohort to determine mode of transport to school, time taken to get to school and engagement in specific physical activities such as playing football, running, cattle herding, playing netball, swimming, fetching water from streams and fetching firewood.

7.2.2: Protocol and indices of weight status:

Habitual physical activity was monitored by accelerometry for the child cohort as detailed in Chapter 6. For the adolescent cohort, subjects were monitored for 4 consecutive school days and 1 weekend day as determined in Chapter 3. Prior to testing of each subject, activity monitors were tested and fully charged. The accelerometer was placed in a small nylon pouch and firmly adjusted at the right hip of the subject by an elastic belt. Activity diaries were completed by each subject or their parent(s). BMI was determined relative to International Obesity Task Force (IOTF) (Cole *et al*., 2000) which developed definitions of overweight and obesity based on BMI centile curves that passed through the adult cutoff points of BMI 25 and 30. The resulting set of age and gender specific BMI cutoff points for children and adolescents was then used to define subjects as overweight or obese, and BMI z scores were calculated relative to WHO reference data (WHO, 2007a, WHO, 2007b) for children and adolescents.

7.2.3: Statistical analysis:

Descriptive statistics included calculations of means, standard deviation and test of group differences following a Shapiro Wilk test for normality. Differences between genders and between children and adolescent cohorts in physical characteristics and indices of adiposity were tested by Generalised Linear Models. Compliance to published public health guidelines for appropriate physical activity levels of accumulating at least 60 min of MVPA daily (CMO, 2011) was also assessed. Multiple stepwise regression analysis was conducted to assess the effect of environment (Centre for child sample) or (rural vs. urban for adolescent sample) on main study outcomes (i.e., time sedentary, total physical activity in CPM, time in MVPA and BMI z score) controlling for age, gender, and environment. In addition, Generalised linear regression models were also explored with environment (Centre for child sample) or (rural vs. urban for adolescent sample), sex and age as covariates to determine whether there was an effect of environment, independent of physical activity, on adiposity in children and adolescents. Compliance to published public health guidelines for appropriate MVPA levels was also assessed using binary logistic regression analysis, with MVPA compliance (coded as 0: <60 min, 1: >60 min) as the dependent variable. The predictors were environment and sex, and their interaction. Statistical significance was declared at $P<0.05$. All statistical analysis was completed using the software package SPSS, Version 17.0 (SPSS, inc., Chicago, IL).

7.3 Results

7.3.1 Descriptive data:

Descriptive characteristics including anthropometric and key physical activity variables are shown in Table 7.1 and Table 7.2. Adolescent subjects engaged in significantly ($P<0.05$) higher volumes of physical activity compared to children: average CPM 753 ± 237 vs. 581 ± 118 for adolescent and child subjects respectively (Table 7.1, Table 7.2). Similarly, adolescent subjects engaged in significantly more MVPA compared to children: 75 ± 29 vs. 55 ± 18, for adolescent vs. children respectively (Table 7.1, Table 7.2). However, there was no significant difference in time spent sedentary between adolescents and children: 379 ± 68 vs. 394 ± 53, for adolescent vs. children respectively.

In adolescents, analysis of the physical activity diaries of rural subjects revealed that all 97 individuals reported either walking (n = 39 or 40%) or running (n = 58 or 60%) to school, covering distances ranging from <1 km to >7 km. Forty eight % (n = 47) of rural subjects reported taking less than 30 min to get to school, 21% (n = 20) between 30 min to 1 h to get to school, while the remaining 31% (n = 30) of these subjects reported taking more than 1 h to get to school. On the other hand, 50% (n = 52) of the urban subjects reported that they travelled to school by car while 41% (n = 42) walked to school. Only about 9% (n = 9) of the urban subjects reported running to school. All the urban subjects who either walked or ran to school reported that it took them less than 30 min to get to school (Table 7.2). All 200 adolescent subjects reported that they were involved in household chores and leisure time was spent doing active physical activity such as running, jumping, skipping, playing football or netball, swimming and sedentary activities such as study, watching television or listening to the radio. In addition, rural adolescents typically reported engaging in activities not reported often by the urban adolescents: gardening, fetching water from streams, fetching firewood and cattle herding.

Table 7.1: Descriptive characteristics of child participants (mean ± SD).

	Males	Females	All
UGENT	11	11	22
UGLW	9	7	16
UZAZ	10	12	22
All	30	30	60
Age (y)	6.7 ± 1.6	7.3 ± 1.3	6.9 ± 1.5ϕ
Height (m)	1.19 ± 1.0	1.24 ± 0.8	1.21 ± 0.1ϕ
Weight (kg)	24.4 ± 7.6	25.1 ± 5.4	24.7 ± 6.6ϕ
BMI (kg/m^2)	16.9 ± 3.1	16.2 ± 2.8	16.6 ± 3.0
BMI z score	0.3 [-0.7 - 4.2]	0.7 [-0.3 - 5.4]	0.5[-0.3 - 5.4]ϕ
CPM	624 ± 98	543 ± 122†	581 ± 118ϕ
Sedentary (min)	396 ± 51	393 ± 56	394 ± 53
Light activities (min)	268 ± 43	281 ± 49	275 ± 46
Moderate activities (min)	46 ± 12	34 ± 9†	40 ± 12
Vigorous activities (min)	18 [9 - 32]	12 [1 - 31]†	15 [1 - 32]ϕ
MVPA (min)	64 ± 15	46 ± 15†	55 ± 18ϕ
% Sedentary	54 [40 - 66]	55 [41 - 73]	55 [40 - 73]
% Light	37 [27 - 47]	39 [24 - 52]	38 [24-52]
% Moderate	6 [4 - 9]	5 [2 - 7]	5 [2 - 9]
% Vigorous	3 [1 - 5]	2 [0 - 4]	2 [0 - 5]
% MVPA	9 [5 - 13]	6 [3 - 10]	8 [3 - 13]
% Complaince	50	20†	35ϕ

CPM - Counts per min

† - Significant difference between males and females

ϕ - Significant difference between child and adolescent cohorts

BMI - Body mass Index

MVPA - Moderate to Vigorous Physical Activity

% Compliance to published guidelines for appropriate MVPA levels, i.e. an accumulation of a minimum of 60 min MVPA daily

UGENT - University of Ghent UGLW - University of Glasgow

UZAZ - University of Zaragoza

TABLE 7.2: Descriptive characteristics of adolescent participants (mean ± SD).

	All	Rural		Urban	
		Male	Female	Male	Female
Subjects	200	47	50	52	51
Age (years)	13.0 ± 1.0	13.3 ± 0.7	13.3 ± 0.6	13.0 ± 1.0	12.8 ± 0.7
Height (m)	1.60 ± 0.1	1.65 ± 0.1	1.61 ± 0.1	1.57 ± 0.1	1.58 ± 0.1
Weight (kg)	45.0 ± 9.0	41 ± 8.5	42.1 ± 7.3	48.1 ± 8.5	50.0 ± 6.3
BMI ($kg \cdot m^{-2}$)	18.0 ± 3.0	15.0 ± 2.0	16.2 ± 2.5	19.4 ± 2.8*	20.0 ± 2.0‡
BMI z score	-0.5[-3.9- 7.5]	-1.4 [-3.8-1]	-1.3 [-3.9-0.7]	0.1[-0.8-7.5]*	0.3[-0.8- 2.8]‡
Transport to school (% car/walk/run)	26/41/34	0/19/81	0/40/60	50/39/12	51/43/6
CPM	753 ± 237	956 ± 143	915 ± 161	626 ± 151*	537 ± 150‡
Sedentary (min)	379 ± 68	335 ± 42	342 ± 59	391 ± 59*	433 ± 60‡
Light activities (min)	181 ± 45	206 ± 40	199 ± 42	171 ± 44	154 ± 33
Moderate activities (min)	49 ± 19	65 ± 16	60 ± 17	41 ± 15	34 ± 9
Vigorous activities (min)	26 ± 14	35 ± 13	30 ± 12	25 ± 11	18 ± 13
MVPA (min)	75 ± 29	101 ± 21	90 ± 26	66 ± 22*	52 ± 19‡
% Sedentary	60	53	55	63	68
% Light	28	31	31	27	24
% Moderate	8	10	9	6	5
% Vigorous	4	6	5	4	3
% MVPA	12	16	14	10	8
% Compliance	67	100	96	48	24

‡ Significant difference between rural and urban females
* Significant difference between rural males and urban males
BMI: Body mass Index
MVPA: Moderate to Vigorous Physical Activity
CPM: Average count per min
% Compliance to published guidelines for appropriate MVPA levels, i.e. an accumulation of a minimum of 60 min MVPA daily

7.3.2 Comparison of physical activity levels and sedentary behaviour in European children and Kenyan adolescents from rural and urban areas:

In children, total physical activity assessed as average CPM was significantly higher in male vs. female subjects (624 ± 98 vs. 543 ± 122 CPM, respectively, $P<0.05$). Similarly, males spent more time in MVPA compared to the females (64 ± 15 vs. 46 ± 15 min, respectively, $P<0.05$) (Table 7.1). There was no significant environmental influence on physical activity in children. In adolescents on the other hand, average CPM was significantly higher in rural male vs. urban male subjects (956 ± 143 vs. 626 ± 151 CPM, respectively, $P<0.05$) (Table 7.2). Similarly, average CPM was significantly higher in rural female vs. urban female subjects (915 ± 161 vs. 537 ± 150 CPM, respectively, $P<0.05$) (Table 7.2). With regard to patterns of activity and sedentary behaviour, urban males spent significantly more time sedentary than rural males (391 ± 59 vs. 335 ± 42 min, respectively, $P<0.001$) (Table 7.2). Similarly, rural males spent more time in MVPA compared to the urban males (101 ± 21 vs. 66 ± 22 min, respectively, $P<0.05$) (Table 7.2). Similar patterns of activity and sedentary behaviour as those reported in males were found in females. As such, there were significant differences in the daily time spent in sedentary behaviour between rural female vs. urban females (342 ± 59 vs. 433 ± 60 min, respectively, $P<0.001$) (Table 7.2), rural females spent more time in MVPA compared to urban females (90 ± 26 vs. 52 ± 19 min, respectively, $P<0.05$) (Table 7.2).

In the child sample, mean BMI z scores was 0.5 [-0.3 - 5.4], median [range]. However, mean BMI z scores were significantly ($P<0.05$) lower in males compared to female subjects (0.3 [-0.7 - 4.2] vs. 0.7 [-0.3 - 5.4] for males vs. females respectively, $P<0.05$) (Table 7.1). Furthermore, 2 male and 2 female subjects were overweight and 2 male and 4 females subjects were classified as obese according to the IOTF definitions (Cole *et al*., 2000). The prevalence of overweight/obesity was therefore estimated as approximately 17% (i.e. 10 out of 60) in children. In the adolescents on the other hand, mean BMI z score was significantly ($P<0.05$) lower in rural male compared to urban male subjects (-1.4 [-3.8 - 1 vs. 0.1 [-0.8 - 7.5] for rural vs. urban males respectively, $P<0.05$) (Table 7.2). Similarly, BMI z scores were significantly ($P<0.05$) lower in rural female compared to urban female subjects (-1.3 [-3.9 - 0.7] vs. 0.3 [-0.8 - 2.8] for rural vs. urban females respectively, $P<0.05$) (Table 7.2). Furthermore, none of the rural adolescents in the present study were classified as either overweight or obese according to the IOTF definitions (Cole *et al*., 2000). On the other hand, 3 male and 6 female urban subjects were classified as overweight and 1 urban male was classified as obese. The prevalence of overweight/obesity in adolescent subjects was therefore estimated as 5% (i.e. 10 out of 200).

7.3.3 The relation between physical activity, sedentary behaviour and BMI z scores in children and adolescents:

In children, BMI z score was positively related to time engaged in sedentary activities ($r = 0.27$, $P< 0.05$). However, there was no association between time engaged in either light, moderate, vigorous or MVPA and BMI z score. In adolescents on the other hand, BMI z score was negatively correlated with sedentary time ($r = ^{-} 0.18$, $P<0.05$) but positively correlated with time engaged in light ($r = 0.16$, $P< 0.05$), moderate ($r = 0.18$, $P< 0.05$), vigorous ($r = 0.17$, $P< 0.05$) and MVPA ($r = 0.2$, P, 0.05).

7.3.4 Multivariate analysis of the impact of environment, physical activity and sedentary behaviour on indices of adiposity in children and adolescents:

In order to identify the factors that may be associated with BMI z score, objectively measured physical activity, sedentary behaviour and the environment in the present study, total volume of physical activity (as evaluated by average CPM) and intensities of physical activity and sedentary behaviour (time sedentary, time in MVPA) were assessed by multivariate regression analysis. The regression model included environment (centre - for child sample) (urban vs. rural - for adolescent sample), gender and age. In children, BMI z score was significantly ($P<0.05$) influenced by sedentary time (adjusted $R^2 = 0.06$) with a total model R^2 of 0.07. However, when sedentary time was controlled, only gender was significantly associated with BMI z scores $F\ (7, 54) = 1.05$, $P<0.05$). Furthermore, environment was not associated with BMI z scores ($P>0.05$). In addition, environment was not significantly associated with sedentary time or MVPA ($P>0.05$). In adolescents on the other hand, BMI z score was significantly ($P<0.05$) influenced by environment (adjusted $R^2 = 0.51$) with a total model R^2 of 0.47. Similarly, total volume of physical activity was significantly ($P<0.05$) influenced by environment (adjusted $R^2 = 0.58$) with a total model R^2 of 0.59. In addition, time sedentary was influenced significantly ($P<0.05$) by environment (adjusted $R^2 = 0.60$) with a total model R^2 of 0.61. Furthermore, time in MVPA was significantly ($P<0.05$) influenced by environment (adjusted $R^2 = 0.30$) with a total model R^2 of 0.31. In addition, BMI z score was significantly influenced by environment independent of sedentary time, $F (5, 194) = 35.17$, $P<0.05$), total volume of activity (CPM) $F (5, 194) = 35.09$, $P<0.05$) or time engaged in MVPA $F (5, 194) = 34.97$, $P<0.05$). Thus, in children, gender had an effect on BMI z score regardless of environment, physical activity levels and age. In adolescents on the other hand, environment had a clear effect on BMI z score, regardless of physical activity, gender and age.

7.3.5 Compliance with recommended MVPA levels in children and adolescents:

In adolescents, approximately 67% (i.e. 134 out of 200) of all subjects met the current public health guidelines for MVPA, defined as an average of $\geq$60 min per day of MVPA over the days of monitoring compared to only 33% (i.e. 20 out 60) in children. However, a greater percentage of rural males (100% or 47 out of 47) compared to urban males (48% or 25 out of 52) fulfilled this recommendation. Similarly, a greater percentage of rural females (96% or 48 out of 50) compared to urban females (24% or 12 out 51) fulfilled this recommendation. Thus, in adolescents, environment was the only significant predictor of MVPA compliance (odds ratio = 50.6 (95% CI 18.1 - 185, $P<0.05$). On the contrary, gender was not a significant predictor, nor was there significant gender by environment interaction ($P>0.05$). However, in children, gender was the only significant predictor of MVPA compliance (odds ratio = 71.4 (95% CI 21.1 - 108, $P<0.05$), environment and age were not significant predictors of MVPA compliance ($P>0.05$).

7.4 Discussion

In the present study there were marked differences in habitual total volume of physical activity (CPM), MVPA, sedentary time and weight status (reflected here as BMI and BMI z score) between children and adolescents and between rural and urban adolescents studied. In children, sedentary time partially explained the differences in BMI z scores. However, when sedentary time was controlled, gender was the principal determinant of BMI z scores. In adolescents on the other hand, there were differences in indices of adiposity that were significantly explained by differences in engagement in sedentary and physical activities of different intensities but were not explained by differences between the urban and rural groups in other variables such as age and gender. However, when differences in physical activity profiles between rural and urban subjects were controlled, the differences in indices of adiposity persisted. This is consistent with the idea that some of the rural vs urban differences in BMI may result from differences in nutrition or any other relevant factors beyond physical activity, age and gender.

Previous studies on Kenyan adolescents from rural areas which utilised subjective measures of physical activity indicated a highly active lifestyle with subjects reporting that they walked on average for 3 h/d and also spent an average of 40 min/d working in the field (Larsen *et al*., 2004, Onywera *et al*., 2006). Rural adolescents consistently reported spending part of their 'leisure time' (defined here as time away from school), engaged in physically active household chores. For example, subjects reported activities such as fetching water from distant streams and fetching firewood, gardening, and running involved in cattle herding. In contrast, the urban sample studied either did not report such activities or reported these activities very infrequently. The urban adolescent subjects reported that they spent their leisure time pursuing largely sedentary activities such as studying, watching television and listening to the radio. The differences in reported physical activity profiles between rural and urban adolescents are an indication of the distinct socioeconomic and built environments between these two groups.

Rural areas in Kenya do not have modern amenities such as mains electricity or piped water and schools are located remotely from villages which may help explain the more active lifestyle in the rural group in the present study. On the other hand, urban schools and households are served with modern necessities such as piped water and mains electricity with accessible roads and frequent motorised transportation. Consequently, urban adolescents might be expected to spend less of their leisure time engaged in such physically active chores. Previous studies which relied on subjective evaluation of physical activity in Kenyan adolescents (Larsen *et al*., 2004, Onywera *et al*., 2006) also suggested that urban vs. rural differences might exist in Kenya. While the significant differences between urban and rural groups in the objectively measured physical activity in the present study might have been 'expected' based on the aforementioned studies and the lifestyle differences recorded in the diaries, differences in subjectively measured habitual physical activity and sedentary behaviour are not always supported by objective measures. In the UK for example, studies which use subjective methods consistently find socioeconomic differences in child and adolescent physical activity and sedentary behaviour which are consistently not found when physical activity is measured objectively (Kelly *et al*., 2006).

Direct comparisons of urban vs. rural differences in objectively measured habitual physical activity and sedentary behaviour in adolescents especially from Sub Saharan Africa are rare and therefore there are no directly comparable studies. Only one study appears to have measured habitual physical activity objectively in Sub Saharan children and adolescents and compared this with levels in Portuguese children and adolescents (Prista *et al*., 2009). It was found that Mozambican children and adolescents had higher levels of vigorous physical activity compared to their Portuguese counterparts (31 and 46% respectively for boys and girls from Portugal, and 57 and 64% respectively for boys and girls from Mozambique. However, the Portuguese group engaged in significantly higher frequency and duration of MVPA bouts compared to the Mozambican group. These observed differences in physical activity parameters were attributed to environmental influences (Prista *et al*., 2009). On the other hand, Kelly and colleagues (2005) found increased sedentary behaviour and gender differences in rural Irish children aged 4 - 5 y with boys significantly more active than girls (824 vs. 628 CPM) which was higher than levels observed in urbanised children in this study: boys and girls (624 vs. 543 CPM) respectively. On the other hand, Kenyan adolescents from rural areas had higher levels of physical activity and were less sedentary compared to the Irish and European children in this study but showed similar gender differences with boys significantly more active than girls (956 vs. 915 CPM).

In the present study the prevalence of overweight/obesity in children was estimated to be 17% compared to 5% in adolescents. However, considering the influence of the environment on overweight/obesity status in adolescents, 10% of urban adolescents were overweight/obese where as none of the rural subjects were overweight/obese. Similarly , an earlier study reported the prevalence of overweight/obesity in young children in urban areas in Kenya to be twice that found in rural areas (6.2% vs. 3.2%, respectively) suggesting that overnutrition of young children could be an emerging phenomenon in urban Kenya (CBS, MOH & ORC Macro, 2004).

Similarly, prevalence of overweight and obesity was much higher in the urban adults (38% vs. only 18% in the rural adults) (CBS, MOH & ORC Macro, 2004). Therefore, urban populations in Kenya are affected by the nutrition transition (FAO, 2010), but the present study suggests that the influence of the nutrition transition on Kenyan adolescents needs to be explored further.

In children, 33% of subjects met current recommendations of appropriate MVPA levels. In addition, males had a higher compliance compared to females (50% vs. 20%) which was consistent with findings in adolescents. Though, current public health guidelines for physical activity in children and adolescents were not intended specifically for the developing world, it was of interest to assess compliance with the recommendation that adolescents accumulate at least 60 min of MVPA daily. In the present study, a higher percentage of rural adolescents 98% (95 out of 97) averaged 60 min of MVPA per day compared to only 34% (17 out of 103) of the urban sample (Table 7.2). Interestingly, urban Kenyan adolescent compliance levels were similar to compliance observed in the urbanised European children in this study. This differences are consistent with the significant differences in objectively measured habitual physical activity between the groups noted above and supportive of the lifestyle differences (such as differences in active commuting to school) which were revealed by the activity diaries, anecdotal reports of rapid urbanisation of rural Kenya and the increasing trend towards sedentary behaviour in children and adolescents especially from urban areas.

The use of accelerometry permitted objective and precise measurement of habitual physical activity and sedentary behaviour over long periods of time, and this has been rare in studies of very young children and adolescents in developing countries to date. Testing for associations between objectively measured physical activity and sedentary behaviour and indices of adiposity especially in Sub Saharan Africa has been even less common. However, the cross sectional study design makes it uncertain that lower levels of physical activity and/or higher levels of sedentary behaviour in the urban group actually caused higher adiposity. On the other hand, a previous study suggested that differences in energy intake and not physical activity levels may primarily explain observed differences in adiposity in adults (Westerterp & Speakman, 2008) but dietary intake was not measured in the present study. Generalised linear modelling suggested that other factors beyond physical activity and age were important predictors of adiposity and therefore the impact of energy intake needs to be determined in both cohorts. Secondly, it was impossible to account for possible seasonal variation in habitual physical activity levels and sedentary behaviour in children and adolescents in the present study, yet seasonal variation might either increase or decrease habitual physical activity and sedentary behaviour differences between groups. In summary, the present study found marked differences in daily physical activity volumes and intensities between children and adolescents and also between rural and urban adolescents in Kenya, with much lower levels of physical activity and higher levels of sedentary behaviour in the urban groups. In addition, rural adolescents had much lower indices of adiposity compared to urban adolescents, and higher adiposity was associated with both lower levels of physical activity and higher levels of sedentary behaviour. On the other hand, controlling for differences in physical activity and sedentary behaviour in children and adolescent indicated that, in children gender, whereas in adolescent environment were significant determinants of adiposity.

CHAPTER 8

8.0 General Discussion

This thesis was designed to examine physical activity in two distinct cohorts with greatly different physical activity levels. Through the chapters of this thesis, a number of research questions have been considered relating to assessing the impact of methodological issues in acclerometry, validating uniaxial and triaxial accelerometers and application of validated accelerometer methodology to assess the impact of physical activity and the environment on energy expenditure and indices of adiposity in subjects from very distinct environments (i.e. European children and Kenyan adolescents). Firstly, the impact of choice of epoch and cutpoint selection on accelerometer outcome variables was determined. In addition, the reliability of accelerometer outcome variables over several days and hours of measurement was also assessed in children and adolescents. Secondly, the predictive validity of uniaxial and triaxial accelerometers was examined during structured activities in adolescents against indirect calorimetry and also during free living activities in children and adolescents against DLW. Finally, the validated uniaxial accelerometer (The ActiTrainer (ActiGraph LLC, Pensacola, FL, USA)) was used to assess the impact of variable engagement in physical activity on energy expenditure and adiposity in children and adolescents. Furthermore, the impact of the environment on physical activity and adiposity was also assessed. The main findings described in this thesis are: (a) that 15 s epoch reports significantly higher engagement in physical activity compared to a 60 s epoch in both the children and adolescents cohorts (b) choice of cutpoints significantly affected classification of physical activity and sedentary behaviour in both cohorts (c) a minimum of 6 h for 7 - 9 days in the cohort involving children and a minimum of 6 h for 4 - 5 days in the adolescents were required to reliably measure physical activity (d) triaxial accelerometry reported better predictive validity compared to uniaxial accelerometry during structured activities. In addition, HR monitoring did not improve the predictive validity of either accelerometer during structured activities (e) during free living activities, however, uniaxial and triaxial accelerometry reported comparable predictive validity. The addition of HR monitoring improved the predictive validity of the uniaxial accelerometry by approximately 4% in both cohorts (f) in both cohorts, total volume of physical activity and patterns (time engaged in light and moderate to vigorous physical activity) were significantly associated with energy expenditure (Δr^2 ranging from 14 - 20%) (g) physical activity and sedentary behaviour were significantly related to indices of adiposity in both cohorts (adjusted R^2 ranging from 0.06 - 0.6) (h) the environment was a significant predictor of physical activity and indices of adiposity in the adolescents but not in children. The findings of this thesis have important implications on strategies to standardise accelerometry field protocols and future studies on the association between physical activity, the environment and health outcomes.

Recent progress in accelerometer processing, such as the ability to sample physical activity data using 1 s epoch for extensive periods of time, combination of accelerometers with other physiological parameters such as HR offer promise for increased accuracy of physical activity estimates from body worn accelerometers, precision that could potentially improve the ability to observe significant relationships with health outcomes beyond those observed in the current studies (Gabriel *et al.*, 2010). However, several issues on epoch length still remain unclear. Firstly, it is not known which epoch length produces the most accurate estimate of actual physical activity performed. To address this issue, comparison of time spent in rest, light, moderate, vigorous and MVPA against a criterion measure such as direct observation needs to be examined (Edwardson & Gorely, 2010). However, direct observation of free living subjects for extensive periods of time is a challenge that remains to be surmounted. A possible approach would be to combine accelerometry with closed circuit television (CCTV) surveillance cameras for extensive monitoring. In support of the current debate on the most appropriate epoch length for accurate assessment of sedentary behaviour and physical activity in paediatric and adolescents studies, the findings (chapter 3) indicated that using 15 s epoch reported 8 - 82 min in children and 7 - 51 min in adolescents more time in sedentary activity compared to 60 s epoch. Similarly, using a 15 s epoch reported 9 - 13 min in children and 9 - 19 min in adolescents more time in MVPA compared to 60 s epoch using several published cutpoints. These findings could be interpreted to indicate that lower epoch durations are more appropriate for optimal assessment of physical activity. This is consistent with the finding that high frequency sampling of physical activity appears more accurate and reliable in quantifying physical activity (Dorsey *et al*., 2009 and therefore, underestimation of time spent in high intensity activities when sampling physical activity using long epochs might mask the relationship between physical activity and health (Turner & Robling, 2003, Rowlands *et al*., 2006).

The imprecise detection of true physical activity levels by using longer epoch durations increases the risk for non differential misclassification, which can result in a reduction in the strength of association between physical activity and health outcomes of interest (Gabriel *et al*., 2010). It is intuitive that capturing activity in shorter epochs would result in a more precise measurement of overall physical activity based on the findings that 15 s epoch reported more time in sedentary activity and MVPA (Chapter 3). However, the actual physiological significance needs to be determined. Therefore, the optimal epoch length to use in the assessment of physical activity would appear to be 15 s. On the other hand, intensity thresholds for moderate and vigorous intensity physical activity derived specifically for use in young people vary from between 615 - 3200 counts/min, even with the same accelerometer model (Puyau *et al*., 2002, Basquet *et al*., 2007, Metallinos-Katsaras *et al.,* 2007). Using these different thresholds on the same data, it is possible to show that the same group of subjects are either inactive or sufficiently active (Reilly *et al*., 2008) defined as accumulating at least 60 min of MVPA per day (CMO, 2011). Recently, Trost and colleagues (2011) assessed the ability of several published accelerometer cutpoints for children and adolescents to correctly classify sedentary, lifestyle, and ambulatory activities. These authors recommend use of the Evenson ActiGraph cutpoints to estimate time spent in sedentary, light, moderate, and vigorous intensity activity in children and adolescents (Trost *et al*., 2011). Incidentally, the Evenson cutpoints indicated the greatest difference in reported time in sedentary and MVPA when comparing 15 s vs. 60 s epoch compared to all the other cutpoints (Chapter 3). Therefore, the current evidence indicates that the Evenson cutpoint are the most appropriate to use in accelerometer studies using ActiGraph accelerometer.

Minimum daily wear time is a critical data reduction issue, because it affects the proportion of files that can be included in analyses (Table 3.3). In children and adolescents subject compliance declined with increased monitoring period. This was more drastic in active Kenyan adolescents compared to European children (i.e. 52% vs. 12%) in children vs. adolescents, respectively. The poor compliance in Kenyan adolescents was mainly due to the novelty of accelerometry in Africa and the remoteness of some of the villages which made it difficult to supervise subject compliance. The minimum must be high enough to eliminate days when the monitor was clearly not worn long enough to accurately depict physical activity but low enough to prevent too many days from being eliminated, which would bias the sample and reduce sample size and statistical power (Colley *et al*., 2010). Calculation of wear time is complicated by the fact that inactivity is part of normal behaviour. Therefore, instead of simply deleting zero count values from the dataset, it is necessary to apply a decision rule that allows for a certain number and pattern of consecutive zeros throughout the day in order to capture and assess true inactivity. This is referred to as the ‘allowable interruption period’ and ranges from 10 to 60 minutes (Màsse *et al.,* 2005, Colley *et al.,* 2010). In this thesis, the allowable interruption periods was ≥20 min of consecutive zeroes as determined by Treuth *et al* (2003) who observed that this period of inactivity was not compatible with monitor wear in children and adolescents. In addition, a calculation of reliability estimates of accelerometer outcome variables is indicated in attempting to assess the robustness of these variables over several days of monitoring in specific samples. In children 7 - 9 days, whereas in adolescent 4 - 5 day including at least one weekend day were the minimum required to reliably assess physical activity. Seven days of continuous monitoring seem logical but because protocol adherence tends to decrease with days of wear, it may be more practical to balance reliability with feasibility (Matthews *et al.,* 2012). Monitor malfunctions can and do occur. Therefore, it is essential that researchers continually check the reliability of the measurement tool itself (Colley *et al*., 2010). Regular calibration is typically required to achieve optimum performance in most devices used for scientific measurement. This ensures that measurements both within and between devices are consistent over the course of many experiments. Thus, before commencement of the studies in this thesis all accelerometer devices were sent to the manufacturers for recalibration. However, few studies have examined the technical reliability of accelerometers, largely because of the lack of commercially available calibration units (Colley *et al*., 2010). Therefore screening spurious data is always warranted to ensure that the various outcome variables are not contaminated by extreme values such as negative counts that are likely a result of faulty units (Esliger *et al*., 2005). On the other hand, the spurious data threshold of 15,000 CPM (recommended for the ActiGraph) is too low (Colley *et al*., 2010). For instance, high performance runners or children engaged in jumping games such as playing on the trampoline could legitimately accumulate accelerometer counts close to 20,000 CPM (Colley *et al*., 2010). Unless such data are captured, the level of activity of these participants would be underestimated (Colley *et al*., 2010). Therefore, the threshold for defining spurious data must be low enough to exclude incorrect high values (for example, monitor aberrations) but high enough to include legitimate values that reflect vigorous activity (Colley *et al*., 2010). In comparison to the 3DNX (BioTel Limited, Bristol, UK) the actiGraph accelerometers have been extensively used in physical activity research because of their small size, non intrusion in free living conditions, and good evidence of validity and acceptability for long term use in other populations (Rothney *et al*., 2008b). Collectively, ActiGraph monitors have been in use for more than a decade and have undergone several hardware and software revisions (Rothney *et al*., 2008b). These monitors have the greatest body of consistent and high quality evidence to support their use (De Vries *et al.,* 2006, Reilly *et al.,* 2008). In Chapter 4, the novel ActiTrainer and GT3X ActiGraph accelerometers were found to be valid for the assessment of physical activity and $\dot{V}O_2$ based on the linear increase in accelerometer outputs with increasing physical activity intensity and $\dot{V}O_2$. Furthermore, 40 - 55% of the variance in $\dot{V}O_2$ could be explained by ActTrainer and GT3X outputs respectively. In addition, when jumping was not included in the protocol of activities, the fit of the regression models increased significantly (P<0.05) to 76% and 80% for the ActiTrainer and GT3X accelerometer respectively (Chapter 4). However, a known limitation with uniaxial (vertical) accelerometers is that non ambulatory activities that do not involve vertical movement of the trunk (when waist mounted) such as cycling are underestimated (Corder *et al*., 2007b), which may not be a problem in cohorts that do not spend significant amounts of time cycling such as the adolescent cohort in this thesis. Additionally, the plateau of uniaxial accelerometer outputs at fast running speed limits its usefulness in assessment of vigorous activities. This however, may not be a limitation in more sedentary cohorts such as in European children who spent only 2% of the monitored time in vigorous activity compared to 10% in adolescents. Triaxial GT3X accelerometer on the other hand, had a distinct advantage over uniaxial ActiTrainer, since GT3X outputs did not plateau at peak running speed and it generated better fitting regression models (R^2 = 40% vs. 55%) for the uniaxial ActiTrainer vs. triaxial GT3X accelerometer during structured activities (Chapter 4). In addition, ΔHR_{acc} was not associated with energy expenditure during structured activities but was associated with energy expenditure during free-living activities, this may be due to the time frameof observarion. The improvement of fit when HR was added to the model was approximately 4% - this may require a longer period of observation to be noticeable. The choice of activities when calibrating activity counts against energy expenditure likely affects the ability of a prediction equation to estimate accurately energy expenditure under free living conditions (Nilsson *et al*., 2008). In Chapter 4 for instance, removal of jumping activities from the menu of activities used in the prediction equations significantly improved the predictive validity of accelerometers, based on the finding that the relationship between accelerometer outputs and $\dot{V}O_2$ was significantly skewed during jumping activity. However, accelerometer validity improved markedly in assessing ambulatory activities in Kenyan adolescents. Thus, the derived regression equation from the relationship between ActiTrainer outputs and $\dot{V}O_2$ during structured activities (excluding jumping) was used to examine the predictive validity the

ActiTrainer accelerometer during free living activities. ActiTrainer outputs modestly underestimated average TEE by 1.1 MJ/d in comparison to DLW determined TEE (Chapter 4). Therefore, the chosen mixture of activities during calibration will definitely affect the slope and intercept of the regression line for the relationship between activity counts and $\dot{V}O_2$ and therefore limit the overall utility of the prediction equations generated to a narrow range of applications. During validation of accelerometer outputs it is important to account for variability in outputs in subjects performing physical activity of the same intensity. For instance, Welk (2005) when examining the reliability of ActiGraph accelerometer counts during a standardised treadmill protocol found that for a standardised bout of activity, counts can vary by 20% for participants wearing the same monitor and performing the same absolute workload. Furthermore, differences in step frequencies have been reported to explain 11% and 40% of the speed adjusted variance in ActiGraph output in walking and running, respectively (Brage *et al.*, 2003). Therefore, the variability in accelerometer outputs among people doing a certain level of physical activity, likely reflects differences across people in gait, stride length and frequency, positioning of the accelerometer, and technical issues associated with accelerometers (Jago *et al.*, 2007, Moeller *et al.*, 2008) which in the end will contribute to an increased random variation in the field and consequently limit the accuracy of $\dot{V}O_2$ prediction models in this thesis.

During free living activities in children, uniaxial actiTrainer reported comparable validity to triaxial 3DNX (Chapter 5). Furthermore, addition of ΔHR_{acc} monitoring significantly improved the model prediction accuracy by approximately 3 - 5% in both child and adolescent cohorts. However, maximum HR and mean HR were not significantly related to energy expenditure in both cohorts. Estimating energy expenditure using accelerometers has been reviewed systematically by Plasqui & Westerterp (2007), it was reported that the Tracmor and ActiGraph accelerometers correlate reasonably well with DLW measured energy expenditure. However, the relationship between accelerometer outputs and energy expenditure still remains of topical interest based on the large number of accelerometers that have yet to be properly validated. Therefore, the evaluation of the relative validity of accelerometer outputs, that is longitudinal acceleration (uniaxial) and triaxial accelerometer outputs during structured and free living activities in relation to energy expenditure associated with physical activity and sedentary behaviour is useful in determination of the optimal accelerometer outputs associated with physiological outcomes. However, the results from accelerometer validation studies are not always obvious. Theoretically, the highest accuracy for the prediction of energy expenditure is achieved by triaxial accelerometry. However, the study in Chapter 5 indicates that this may not necessarily be the case. For instance the uniaxial ActiTrainer reported comparable predictive validity during the assessment of TEE, AEE and PAL as the triaxial 3DNX based on the observed Δr^2 and SEE values. In general, no one monitor is superior to another and selection depends primarily upon the research interest (Garatachea *et al*., 2010). However, evaluation of the validity of the ActiTrainer, GT3X and 3DNX accelerometers (Chapter 4 and 5) indicated that these devices were valid for assessment of physical activity in predominantly inactive children and relatively active adolescents based on the Δr^2 and SEE values observed in both cohorts (Chapter 4 and 5). In addition, uniaxial and triaxial accelerometer outputs were significant predictors of AEE and PAL in children. It would have been interesting to study the relationship between accelerometer outputs and AEE and PAL in adolescents as well. However, the accuracy of most published BMR prediction equation in Africans of Sub Saharan descent has been questioned (Vander Weg *et al.,* 2004), since most of these equations fail to account for differences in ethnicity and body fat in the predictions. Therefore, there is an urgent need to validate BMR prediction equations in Africans, which would enhance future studies calorimetry studies in Africans. Therefore, the performance of uniaxial ActiTrainer with HR monitoring, triaxial GT3X and 3DNX observed in this thesis needs to be confirmed in other free living populations.

Particular physical activity patterns may be beneficial to health. For example, subjects with the same physical activity level (CPM) might accumulate their physical activity in very different combinations of intensity, duration, frequency and type (Mattocks *et al*., 2008b). Furthermore, there is also developing interest in the concept of 'sedentariness' (Ekelund *et al*., 2006). It is now increasingly accepted that sedentary behaviour is not simply a lack of physical activity but is an independent behaviour (TV/computer use, reading, homework, etc.), which constitutes a potential risk to health irrespective of physical activity level (Ekelund *et al*., 2006) which is consistent with findings in Chapter 6, indicating that sedentary behaviour was independently associated with BMI z scores in children and adolescents. For instance, in children time spent in sedentary activities was negatively correlated with BMI z scores ($r = 0.27$, $P<0.05$). However, there was no association between time engaged in either light, moderate, vigorous or MVPA and BMI z score. Moreover, total volume of physical activity (CPM) and time spent engaged in light or MVPA were significantly associated with energy expenditure in relatively inactive children compared to relatively active adolescent cohorts, respectively (Chapter 6). Increased physical activity, along with the achievement and maintenance of energy balance, has emerged as an important personal health goal for the 21st century (Andre & Wolf, 2007). Consequently, objective assessment of physical activity has gained considerable interest. Thus, easy to wear monitors such as the ActiTrainer, GT3X and 3DNX accelerometers that provide both the patient and the health care providers with objective information about the wearer's lifestyle offer many possibilities to improve the treatment and management of lifestyles diseases (Andre & Wolf, 2007).

The quantitative differences revealed by objective measurement of physical activity in Chapter 7 between children and adolescents (urban vs. rural) groups may therefore reflect marked lifestyle differences which remain between rural and urban subjects. All the rural adolescents in Chapter 7 engaged in active transport to school (i.e. ran or walked to school) where as 50% of the urban adolescents used motorised transport to get to school. In children, BMI z score was significantly influenced by sedentary time. In adolescents, on the other hand, time in sedentary activities as well as in light, moderate, vigorous and MVPA were associated with BMI z score. These findings are broadly consistent with recent systematic reviews on the association between physical activity, sedentary behaviour and adiposity in adolescents (Rey-López*et al*., 2008, Jiménez-Pavon *et al*. 2010). However, there was no association between active commuting and adiposity as reported by Bassett *et al.* (2008) and therefore this association needs to be studied further using objective methods of assessing commuting distance such as GPS as used in this thesis. Lower levels of objectively assessed physical activity were associated with higher indices of adiposity in Chapter 7. Similarly, studies among Western children have shown inverse associations between physical activity and adiposity and other cardiovascular risk markers (Ekelund *et al*., 2004, Andersen *et al*., 2006). Thus, current evidence suggests that contemporary changes in transport, occupations, domestic tasks, and leisure activities have had negative effects on the activity level (Banomi *et al.,* 2010).

In Kenyan adolescents, there were distinct differences in physical activity and indices of adiposity between rural vs. urban adolescents. For instance, a greater percentage of rural males (100% or 47 out of 47) compared to urban males (48% or 25 out of 52) fulfilled the current physical activity recommendation of accumulating at least 60 min of MVPA daily (CMO, 2011). Similarly, a greater percentage of rural females (96% or 48 out of 50) compared to urban females (24% or 12 out 51) fulfilled this recommendation. In addition, none of the rural adolescents in the present study were classified as either overweight or obese according to the IOTF definitions (Cole *et al*., 2000). On the other hand, 3 male and 6 female urban subjects were classified as overweight and 1 urban male was classified as obese. The prevalence of overweight/obesity in rural adolescent subjects was therefore estimated as 0% compared to approximately 10% (i.e. 10 out of 103) in urban adolescents. A number of studies on the 'nutrition transition' have suggested that subjectively measured physical activity, sedentary behaviour, or 'proxies' for physical activity and sedentary behaviour (such as reported TV viewing, mode of transport to school and physically intensive chores), tend to change unfavourably with development and urbanisation in the developing world (Popkin, 2001, Yamauchi *et al*., 2001, Sobngwi *et al*., 2002). Furthermore, the diets of the developing world are shifting rapidly, particularly with respect to fat, caloric sweeteners and animal source foods (Popkin, 2001). This nutritional transition is closely related to and may explain the current demographic and epidemiologic transitions seen in developing countries, with individuals increasingly consuming diets which are higher in energy but low in fibre. These changes are reflected in nutritional outcomes, such as changes in average stature, body composition, and morbidity (Popkin & Gordon-Larsen, 2004). Multivariate analysis in Chapter 7 indicated that when differences in physical activity profiles between rural vs. urban subjects was controlled, differences in adiposity between the two cohorts persisted, suggesting that there may be meaningful differences in caloric intake between these two groups.

In summary, this thesis has found that accelerometry provides an objective, practical, accurate, and reliable means of quantifying amount (volume) and intensity of habitual physical activity and amount of sedentary behaviour in inactive children versus active adolescents. Despite the initial cost and limitations for large scale research applications, accelerometers have been used in longitudinal and cross sectional studies with large samples to assess physical activity levels in children and adolescents (Corder *et al*., 2008). Advances in technology, such as the use of multiple axes accelerometers or their integration with other devices, such as HR monitors or GPS devices, improved field measurement of physical activity and sedentary behaviour in children and adolescents. However, accelerometer use is not without its challenges. These include a lack of understanding on exactly how a monitor functions. For example, proprietary restrictions limit disclosure of which algorithms are used to convert raw acceleration into counts, how to select the most appropriate instrument, standards of monitor wear and field use, how to interpret accelerometer data and how to manipulate and analyse the vast amount of data produced by accelerometry (Trost *et al*., 2005, Ward *et al*., 2005). Additionally, accelerometers cannot capture certain highly static categories of activity or complex movement patterns that combine dynamic and static movements (Matthews, 2005). This is especially true of uniaxial accelerometers, but the inclusion of HR monitoring improved the quality of the data collected during free living activities. Moreover, standardised protocols do not exist for determining the number of monitors that participants should wear the optimal placement on the body, the optimal number of wearing days, or procedures to ensure participant compliance (Corder *et al*., 2008).

This thesis addressed some of these issues and results highlight the increasing need for accurate and objective physical activity field assessment tools such as validated accelerometers. The use of these validated accelerometers continues to show promise in physical activity research based on observed advantages over more traditional physical assessment tools such as questionnaires. Furthermore, it is anticipated that the commercial nature of these instruments will drive an even greater range of features and options in the future, increasing both the opportunity and the challenge of objectively assessing physical activity in children and adolescents (McClain & Tudor-Locke, 2009). Refinements in accelerometry such as combination of motion sensing with other physiological parameters may improve their accuracy for measuring physical activity as seen in Chapter 5, where accelerometry was combined with HR monitoring. Thus, as physical activity monitoring moves into the future, it is incumbent on researchers to be open to new technologies, such as multi sensor arrays, as well as integrating familiar sensors into new devices (Chen *et al*., 2007). Approaches that utilize diverse sensors in a single accelerometer provide more activity information and may be expected to improve the accuracy in physical activity monitoring (Chen *et al*., 2007). Altimeters (pressure sensors) have been used along with an accelerometer to identify movements with altitude changes, such as walking up/downstairs. The ability to classify inclined walking may enhance the accuracy in energy expenditure estimation during physical activity (Yang & Hsu, 2010). Furthermore, the measurements of human heat dissipation, skin temperature and conductivity have also been used in a commercial accelerometer based activity monitor for accurate energy expenditure and metabolism rate assessments (Yang & Hsu, 2010). Accelerometry data obtained from wearable accelerometers can also be synchronised with the activity of daily living (ADL) data recorded by such monitoring systems to better describe the information of human mobility, physical activity, behavioural pattern and functional ability that encompass the important parameters regarding the overall health status of an individual (Yang & Hsu, 2010). Thus, the innovation of integrating physiological measurements with accelerometry may enhance energy expenditure prediction, particularly in sedentary conditions, low intensity activities, activities with limited body movements (e.g. resistance exercise) and even under variable environmental conditions (temperature changes) (Chen *et al*., 2007, Yang & Hsu, 2010). The physical assimilations of multi sensor arrays into portable wireless systems are being achieved in devices such as the Actiheart and SenseWear (BodyMedia). In fact, several cell phone manufacturers are already building activity monitors (accelerometers, gyroscopes, etc.) into cell phones (Chen *et al*., 2007) which may also enhance subject compliance, a major limitation of multi sensor arrays. However, other than a few small studies done with specific activity types and selected populations (such as in children and adolescent during free living activities in this thesis), there have not been significant improvements over their predecessors single accelerometers (as seen in adolescent during structured activities in Chapter 4) and thus more sensors may not necessarily mean better precision (Chen *et al*., 2007). This area needs to be explored further in future studies.

In conclusion, the findings of this thesis are as follow:

a) 15 s epoch using Evenson cutpoints would appear to be the best for use in accelerometer studies in children and adolescents.

b) Reliability of accelerometer variables over several days of measurement is age specific, for young children, at least 7 days and for adolescents, at least 4 days including at least 1 weekend day are required to reliably monitor physical activity.

c) Triaxial GT3X ActiGraph accelerometer was more valid than uniaxial ActiTrainer ActiGraph accelerometer (i.e. R^2 = 48% vs. 34%) for assessment of $\dot{V}O_2$ during structured activities in adolescents. However, addition of HR monitoring did not improve predictive validity of accelerometry during structured activities

d) Uniaxial ActiTrainer and triaxial 3DNX accelerometers report comparable validity during free living activities in children. Furthermore, addition of HR monitoring, improved predictive validity of accelerometry during free living activities in children and adolescents.

e) Total volume of physical activity (i.e. CPM) and physical activity patterns (time in MVPA or light activities) were important determinants of free living energy expenditure in children and adolescents, respectively.

f) Physical activity and sedentary behaviour are associated with indices of adiposity in children and adolescents.

g) The environment and gender are important determinants of physical activity, sedentary behaviour and adiposity in children and adolescents

h) Further studies are required to determine the consistency of these findings in other populations

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