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Comparison Between a Standard Manual and Automated Analysis Of Accelerometer Data and The Effect Methodical Decisions Have On Accelerometer Output

Dissertation, submitted in fulfilment of the requirements for an MSc in Physiology

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Abstract

Background: The impact of accelerometer methodological decisions relating to the assessment of physical activity and sedentary time has not been conclusively determined in young children. With increasing numbers of large scale studies measuring physical activity, it is essential to have a validated method of analysis capable of analysing multiple files at any one time.

Objectives: To describe and compare a standard method of analysis with an automated method of analysis of accelerometer data for use in large scale epidemiological studies. The automated approach also provides investigators with a powerful tool to effectively assess the effects of different decisions/choices on the classification of physical activity and sedentary behaviour by determining 1) the effects of epoch and cut-points on the assessment of physical activity and sedentary time, 2) how to define non wear time and, 3) accelerometer wear time required to achieve reliable accelerometer data in children.

Design: The physical activity levels of 86 children aged 4-10 were measured as part of a larger European study. Children were recruited from centres at Ghent, Glasgow, Gothenburg and Zaragoza.

Methods: Physical activity was assessed for 1 week in 86 children (41 female, 45 male; mean age 7±2 years) by uni-axial accelerometry. The epoch was set at 15 s and re-integrated to 30 s and 60 s. Time spent in sedentary and moderate and vigorous physical activity (MVPA) was assessed using Pate, Puyau, Reilly and Sirard cut points. Non wear time of accelerometer was defined by removal by the 10-, 20-, 30- and 60-mins of consecutive zeros.

Results: There was excellent agreement between the automated method of analysis and accelerometer outputs generated by the standard manual method of analysis. The Reilly cut-

points (<1100 counts/min) indicated less sedentary time per day 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, respectively; P<0.05). Pate cut-points (>420 counts/15 s) reported more MVPA time per day compared to Sirard (890 counts/15 s) and Puyau cut-points (>3200 counts/min) using 15 s epoch: 88 (4-197) mins (median (range) vs. 18 (1-80) mins and 24 (1-100) mins, respectively; P<0.001). Compliance with guidelines of at least 60 mins MVPA was 83%, 77% and 72% for Pate cut-points using 15 s, 30 s and 60 s epoch, respectively but 0% for Sirard and Puyau cut-points across epochs. The number of days required to achieve 80% reliability for counts per minute (CPM), sedentary and MVPA time was 7.4 – 8.5 days.

Conclusion: An automated method of analysis of accelerometer data has successfully compared with manual analysis and should be recommended for use in large scale epidemiological studies. Choice of epoch and cut-points significantly influenced the classification of sedentary and MVPA time and observed compliance to MVPA guidelines, emphasising the need to standardise accelerometer data reduction methods. In order to accurately measure and asses physical activity levels of a population, a uniform analysis must be generated to be able to compare physical activity across populations.

Key words: IDEFICS - Accelerometry - MVPA - objective measurement

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Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Glasgow. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree. Any views expressed in the dissertation are those of the author and in no way represent those of the University of Glasgow. The dissertation has not been presented to any other University for examination either in the United Kingdom or overseas.

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Definitions/Abbreviations

AEE – Activity patterns and Energy Expenditure

BMI – Body Mass Index

CARS - Children's Activity Rating Scale

CPM – Counts Per Minute

DLW – Doubly Labeled Water

DXA - Dual-Energy X-ray Absorptiometry

EE – Energy Expenditure

FATS - (modified) Fargo Activity Time Sampling

ICC - Intra-Class correlation

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

MET - Metabolic Equivalent of Task

MVPA – Moderate to Vigorous Physical Activity

PDPAR - Previous Day Physical Activity Recall

PE – Physical Exercise

SD – Standard Deviation

UGHENT – University of Ghent

UGLW – University of Glasgow

UGOT – University of Gothenburg

UZAZ – University of Zaragoza

Introduction

1.1 Obesity in children

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 per year, of which over 0.3 million per year will be obese children (Jackson-Leach and Lobstein, 2006). Figure 1 shows the annual changes in the numbers of children who are overweight or obese, from 1975 to 2000, and the line is then extrapolated to estimate how high the number could become by 2010. Many studies are also finding the same conclusions.

In Scotland, the Scottish Health Survey (2008) found that according to the BMI of children aged 2-15 year olds, the prevalence of overweight and obesity is on the increase year by year. These increased numbers are particularly high in young boys, the numbers have risen 32.4% in 2003, to 36.1% in 2008. However, the levels of overweight and obese girls has remained at a constant, with no significant difference between the years as 28.9% of girls were classed as being overweight or obese in 2003 and a slight decrease to 26.9% in 2008. According to this health survey, just under a third of Scottish children are obese or overweight on average.

The rise in the occurrence of children being overweight and obese is very concerning as many health risks are associated with obesity. This rise in obesity figures is thought to be due to the changing environment in which children live; with decreased physical activity and poor diets bringing an increase in diet- and lifestyle-related diseases and disorders in children in Europe (Bundred *et al.,*2001). These diet- and lifestyle-related diseases and disorders include; the increased prevalence of overweight and obesity in children, type II diabetes and many cardiovascular risks (Reilly *et al.,*2003). What is particularly concerning about overweight and obese children is that it has been found that a high percentage of obese children remain obese into adulthood (Reilly *et al.,*2003). A recent review by Whitaker *et al.,*(1997) found that 69% of 6-9 year olds in the USA were obese as children, and in this same cohort, 83% of obese 10-14 year olds became obese adults. From these figures it can be seen that adolescent obesity may be more likely to continue into adulthood than childhood obesity, but ultimately it may all begin with childhood obesity.

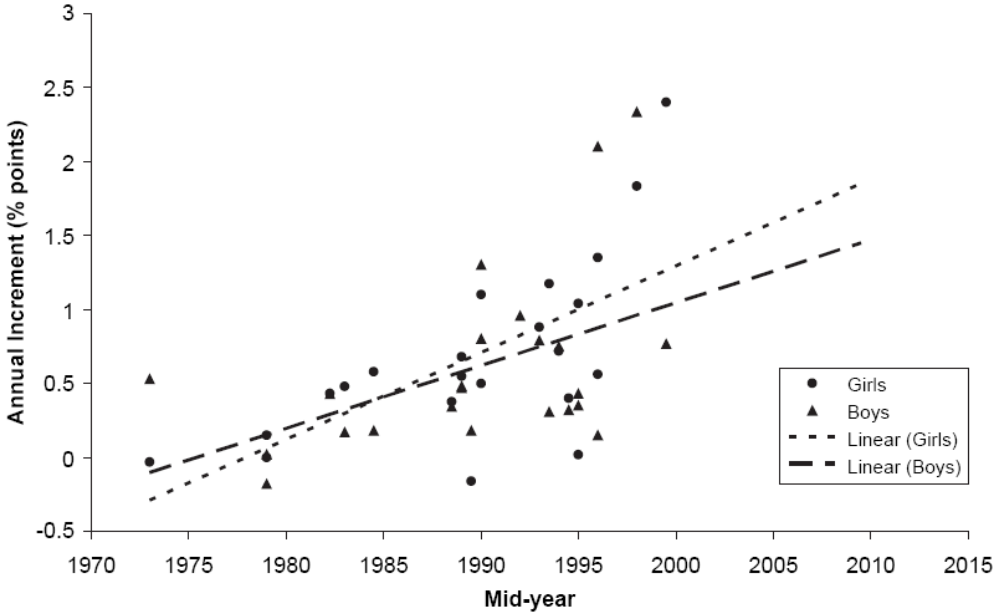


Figure 1: Figure shows the annual prevalence rates of overweight (including obesity) among children, from 1970 to 2010. Taken from Jackson-Leach and Lobstein, (2006).

The current obesity epidemic that the world is facing is due to a negative imbalance between energy expenditure and energy intake (Department of Health, 2004). Children are now less active in day to day life. In the past 20 years the number of children being driven to school has more than doubled, with 30% of school children being driven to school and less than 50% now walking to their schools (Department of Environment, Transport and the Regions, (1999)). Also, within the education curriculum in the UK, physical education (PE) lessons have lessened over recent years and now less time is given to PE in England and Wales than anywhere within Europe (Physical Education Association of Great Britain and Northern Ireland, 1993).

As low levels of physical activity may be a contributing factor to obesity rates in children, the Scottish Physical Activity Task Force is aiming to have all school children partaking in at least 2 hours of "high quality PE (physical exercise) lessons" weekly as part of the 'Let's make Scotland more active: a strategy for physical activity' campaign (2003). This is hoped to be achieved by the Curriculum for Excellence Programme allowing for enough flexibility to ensure that there is time for the provision of at least 2 hours of PE for every child each week. When researching the physical activity levels of obese and non obese children, Trost

et al.,(2001) found that it is actually physical *inactivity* that is an important factor in the occurrence of childhood obesity, rather than measuring how active they are. It was found in this same study that fewer counts were accumulated with the accelerometers in obese children and there were significantly fewer bouts of moderate to vigorous physical activity (MVPA) in obese children compared to those of non obese children. Physical activity guidelines have needed to be created due to the continuing increase in the number of overweight and obese children. These guidelines state that all children should be partaking in at least 60 minutes of MVPA each day in order to receive any health benefits. However, children who are overweight or obese may need to do more than the 60 minutes in order to achieve similar health benefits (Department of Health, 2004). However, it is not believed that children are meeting these current guidelines, so therefore more needs to be done to measure how much activity children are actually taking part in. In order to asses levels of physical activity it is necessary to have reliable and accurate measures of the physical activity levels of children in order to establish the relationship between activity and health and also be able to quantify the frequency and patterns of physical activity within a defined population (Trost *et al.*,2000).

Although the obesity epidemic is regularly associated with low levels of physical activity and high levels of energy intake, it is thought that there is also a genetic component. A large study using 3000 subjects from the Danish adoption register which contained full genetic background details of the biological parents, showed a significantly positive relationship between BMI of adoptee and their biological parents, but no relationship was found between adoptee and adoptive parents. (Stunkard *et al*., 1986) The same Danish group also found a close relationship between adoptee and their biological siblings who were brought up separately. This study suggests that genetics could be a factor in obesity, rather than it being down to the person's environment. However the genetics of obesity is still unclear, with some studies believing that one of the factors in obesity is the environment. The Pima Indians of Arizona have the highest prevalence of obesity and Type 2 diabetes. A study was created to compare the Pima Indians of Arizona to traditional Pima Indians of Mexico. This study found that Pima Indians of Mexico who live the traditional Pima lifestyle were less likely to be obese or have Type 2 diabetes, compared to Pima Indians who lived in developed countries with an affluent lifestyle.

1.2 Measuring Physical Activity in Children

There are many ways to measure physical activity, but it is finding the most reliable, accurate and cost effective method that is difficult. Figure 2 shows the different methods for measuring physical activity, with “Criterion Standards” being the most appropriate method of recording physical activity levels. Each arrow on Figure 2 represents “acceptable criterion standards for the validation of tertiary and secondary level methods” (Sirard and Pate, 2001). Each tier on the model represents levels of reliability, with the top tier being the most stringent. Subjective measures such as self report or interview would not give accurate results if these were the sole measure of physical activity. Accuracy could be improved by combining subjective measures with objective measurements; such as, accelerometers or pedometers with physical activity diaries, which is what the arrows are pointing towards. The middle tier involves subjective measures, which are not entirely reliable on their own. As accurate as the secondary measures are, it is difficult to measure the intensity of the activities performed. By combining subjective measures with doubly labelled water (DLW), it could be possible to compare accelerometer output with caloric output to define a threshold significant to a count number. The most appropriate are thought to be the examples found in the top tier of this model, with direct observation allowing for the subject to be closely monitored each day and night to note their physical activity levels which reduces the issues associated with inaccurate levels of recording of physical activity. The examples in the top tier could be thought of as producing the most reliable results and there is no need to combine with a second method of analysis to record levels of physical activity and/or energy expenditure.

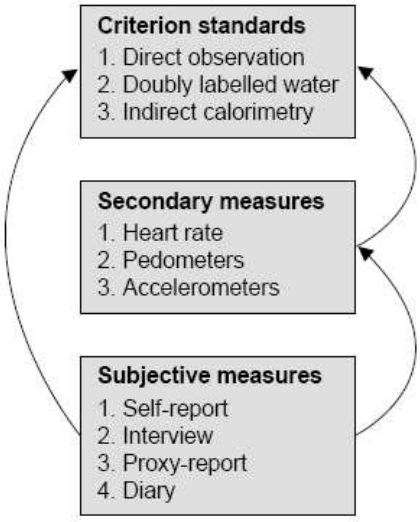


Figure 2: Criterion standards for measuring physical activity. Taken from Physical activity assessment in children and adolescents, (Sirard and Pate, 2001) Each tier represents levels of physical activity measurements, with the arrow representing how to improve accuracy and combining one with the other.

1.2.1 Criterion Standards

The criterion standards displayed in Figure 2 are thought of as being the "gold standard" method of assessing physical activity i.e. through direct observation. There are different observational systems to be used in various settings; examples include. Children's Activity Rating Scale (CARS) (Puhl *et al.*,1990), Modified Fargo Activity Time Sampling survey (FATS) (Bailey *et al.*,1995) and Activity Patterns and Energy expenditure (AEE) (Epstein *et al.*,1984). The CARS measurement involves a large amount of experimenter training before the study begins, with observer training lasting over 8 weeks. During the training the observers watch the children taking part in specific activities at set speeds and rules are made over how to grade these activities or how to measure unexpected activities. Over the 12 month experimental period, there are also weekly discussion meetings to go over any problems experienced. Once the study begins, usually about 7am each day, the child is monitored for approximately 6-12 hours a day. The observers record activity minute by minute, recording at the start of the minute, and mark any changes in activity during that

minute and put the recording into portable computers. As this is quite an intense method of analysis the experimenters rotate in 2 hour shifts throughout the day (DuRant *et al.*,1993).

As can be seen is this brief description of the CARS protocol, there is a very high experimental burden on the experimenter. This is also true for all of the above mentioned direct observational techniques. Some children may not feel comfortable with having the experimenter watching their every move, and this may affect the free-living aspect of the subject. Puhl et al (1990) found that 16.6% of their cohort reacted to the observer being present, which would result in the child perhaps not reflecting their true behaviour patterns. With the recording of activity using these techniques ranging from every 3 seconds to 60 seconds, it is unimaginable to use these methods on large scale studies, therefore more appropriate measures are used to estimate physical activity in these studies.

1.2.2 Subjective Measures

Currently, the most commonly used methods used in large scale studies for measuring physical activity levels in children are subjective techniques (Ward *et al.*,2005). Subjective methods are frequently used due to their low cost and ease of administration (Sallis, 1991). Subjective techniques are those which require a response from the subject partaking in the study, with relatively high participant burden. Again, there are many techniques;
Self report questionnaires - including Previous Day Physical Activity Recall (PDPAR) which involves the subject recalling their activities from the previous day and also recording the intensity of these activities. It has become apparent that children have difficulty in accurately recording their physical activity for periods covering longer than one day (Weston *et al.*,1997). Activity Diaries are used subjects are required to record their daily activities and the intensity levels of these activities. This type of subjective method is considered to be one of the most accurate subjective techniques for adults, but is not very reliable in the paediatric population (Sirard and Pate, 2001). Proxy-reports rely on another person to record the activity (usually a parent or teacher) of each child. These are perhaps not as reliable, as their may be some bias as the recorder may want their pupil/child to appear to be more active and manipulate the results (Whiteman and Green, 1997).

For these reasons it is difficult to use subjective measurements techniques when studying children as it may be difficult for them to recall all of their movements throughout the day, particularly as child play is often random and full of short bursts of activity (Bailey et al. 1995) which increases the difficulty of recall. It would also be hard for young children to recall all of their activity during the day including noting their intensity, duration and frequency of their play (Sirard and Pate, 2001). Subjective methods have also been found to overestimate time spent engaging in physical activity, with the estimated error being between 35 and 50%, varying with age groups and disease conditions (Welk, 2002).

1.2.3. Objective Measures

With subjective techniques perhaps not being the most reliable, objective measures can also be used to measure physical activity. Heart rate monitoring can be used to measure physical activity and energy expenditure (EE) in young children. This type of measurement is dependent on the linear relationship between heart rate and oxygen consumption (Sirard and Pate, 2001). However changes in heart rate are not always due to body movement or intensity of exercise. Factors such as emotional stress, dehydration, increased temperature, caffeine and illness can all cause changes in heart rate without any changes in oxygen consumption (Montoye *et al.*,1996; Melanson and Freedson, 1996; Sirard and Pate 2001). Combining accelerometers with heart rate sensors may be a way of improving this type of research, although there is limited research on this combination. However, a study was completed which used a one piece instrument which measured heart rate and activity. It showed a near perfect agreement when compared to the direct measurement of a room calorimetry which measures energy expenditure under closed rooms where they are supplied with measured air and are given controlled diets and exercises which means that EE can be measured more accurately, including controlled meals given. (Rennie *et al*, 2000). Using this method it can be seen that the elevations in heart rate are due to physical activity and not a response to the environment.

Accelerometer based methods to assess physical activity levels have been shown to be valid, accurate and feasible in large epidemiological studies (Riddoch *et al.*,2004). Accelerometers are small, light and unobtrusive which makes these devices highly suited for use in studies, particularly studies involving young individuals (see Figure 3 and Table 1).

Figure 3: The Actigraph Actitrainer accelerometer device

Transducer	Uni-axis, solid state accelerometer
Dynamic Range	+/- 3G
Dimensions	8.6cm x 3.3cm x 1.5cm
Weight	1.8 oz
Capacity	4MB or 198 Days*
Battery Life	7 Days (Fully Charged, Display On) 14 Days (Fully Charged, Display Off)
Communication	USB 2.0
Resolution	12-bit A/D conversion; 1.46 mG (Raw Data)
Sample Rate	30 Hz
Parameters	Activity, Heart Rate, Steps, Inclinometer, Light
Calibration	Not Required
Water Resistant	Splash

Table 1: Specifications of Actitrainer, taken from manufacturer's website: (http://www.actitrainer.com/products/actitrainer)

Accelerometers are found to produce results similar to the "gold standard" observation techniques, as found by the study by Finn and Specker (2000) who compared the direct observation technique of CARS with the Actiwatch (Mini-mitter Company Inc.) activity monitor. Simultaneous 3-minute mean CARS scores and 3-min activity counts were

recorded over a 6 hour period and then matched for each subject. This study found that the 3 minutes CARS score highly correlated with the 3-minutes activity counts, favouring the use of activity monitors for use in children. A study recruiting subjects through the Avon Longitudinal Study of Parents and Children (ALSPAC) used the Actigraph accelerometer. An equation was developed to predict energy expenditure in peripubertal children from their accelerometer counts. The subjects performed a series of actions (including lying, slow walking, jogging) whilst wearing and Actitrainer accelerometer and a portable metabolic unit (Cosmed K4b^2). This study found that the counts produced by the Actitrainer could successful predict energy expenditure across a mixture of activities, when adjusted for age and gender. These results emphasise the reliability of accelerometers for measuring physical activity and show that they can measure this, perhaps just as accurately as direct observation. Currently, many studies now use accelerometers to measure physical activity in the field (Puyau *et al.*,2002; Nilsson *et al.*,2002; Reilly *et al.*,2003; Pate *et al.*,2006; Jackson *et al.*,2003). Trunk movements produce the greatest amount of physical activity and energy expenditure, and therefore most commercially available accelerometers measure movement in the vertical axis (uniaxial accelerometers), and studies have shown that the accelerometers should be placed on the right hip to improve count accuracy (Reilly *et al.*,2003; Rowlands, 2007).

As mentioned previously, accelerometers are now being incorporated into the design of large scale studies, but there is still an issue of how to analyse such large quantities of data. Although accelerometer use is the preferred method for measuring physical activity, there are some disputes over how to interpret the counts and cut points have been provided which are used to define activity spent at sedentary, light, moderate or vigorous, which is usually achieved by providing a MET (metabolic equivalent of task), where 1 MET is the amount of energy expended at rest (Masse *et al.*,2005). Even though there are a large number of accelerometer validation studies (Freedson *et al.*,2005; Trost *et al.*,1998; Puyau *et al.*,2002), a standardised method of data reduction has not yet been established.

Methodological issues such as identifying minimal wear requirement for a valid day, identifying non-wear time, how to compute outcome variables and how many days of monitoring are enough, all need to be standardised and a consensus met. Another important issue is deciding which epoch to use when designing accelerometer studies has also not yet

been agreed upon. The majority of studies use a setting of 60 second epoch to collect data (Payau *et al.*,2002; Reilly *et al.*,2003,). However other studies believe that a 60 second epoch is too large a time period for measuring the activity of children (Reilly *et al.*,2008). Some studies have suggested that a 60 second epoch might misrepresent intensities by classing activity at a lower intensity, as the epoch is averaged out across the minute's worth of activity, and is therefore perhaps missing the short bouts of high intensity activity which are more common of children (Bailey *et al.*,1995; Reilly *et al.*,2008; Nilsson *et al.*,2002; RP Pate *et al.*,2006). Recently it has been found that epoch lengths as low as 1 and 10 seconds report significantly more time spent in MVPA than when using a longer epoch (Ojiambo *et al.*,2009), and 5 seconds was found to be the most appropriate to detect short periods of intense exercise by children in similar different study (Edwardson and Gorely, 2010)

1.3 An introduction to IDEFICS

As can be seen, there are many available techniques to measure physical activity, but it is still unknown how inactive EU children are. In order to develop an intervention programme which will increase the time spent taking part in physical activity, it is essential to classify the current activity in children. This is particularly important since childhood obesity can continue in to adulthood obesity and lead to related health problems later in life (Reilly, 2006), so if the intervention can be created in childhood it may help to prevent the overweight/obesity issues in later life. In an attempt to counter the epidemic of obese and sedentary children in European children, the IDEFICS (Identification and prevention of dietary- and lifestyle induced health effects in children and infants) study aims to enhance the knowledge of the impact of lifestyle related factors, like physical activity, on children's health. This goal is intended to be achieved by developing, implementing and evaluating specific intervention plans for 2-10 year olds (Bammann *et al.*,2006).

The IDEFICS study is a large multicenter study involving 8 European countries and approximately 16,000 children and is one of the largest single studies to use accelerometers to objectively assess physical activity levels in children. For the purposes of the IDEFICS study, a validation study was carried out in a smaller subgroup of children to compare a number of field measures of body composition and objective measures of physical activity assessment with goal standard reference methods in order to determine the most appropriate

methods of analysis for large epidemiologic and intervention studies such as the IDEFICS study. Here the accelerometer data collected in the validation study is used to discuss the effect of varying accelerometry methodological issues have on accelerometer results. As part of this validation study an automated method of analysis of accelerometer data was to be validated by comparing accelerometer outputs generated by the automated method of analysis with outputs derived by the standard manual method of analysis; and to investigate the impact methodological decisions have on the outcome of the accelerometer analysis. Through developing and strengthening the automated analysis programme R, it is possible to explore these issues.

1.4 Aims of this study

Therefore, the main aims of the study are:

1) To describe and compare an automated method with a standard method of analysis of accelerometer data for use in large scale epidemiological studies

2) To investigate the impact of methodological decisions on accelerometer outcome variable, including:

   - The effects of epoch and cut-point selection on average counts per minute (CPM), sedentary time and MVPA
   - The effect of variation of defining accelerometer non-wear time (removal of consecutive zeros)

2. Methods

2.1 Subjects

The present validation study was part of the large scale IDEFICS study (Ahrens *et al.*,2010) described fully in Bammann *et al.*,2010. Briefly, a total of 98 subjects aged 4 to 10 years old participated in the IDEFICS validation study. Subjects were recruited from 4 different validation centres at the universities of Ghent (UGHENT), Glasgow (UGLW), Gothenburg (UGOT) and Zaragoza (UZAZ). The validation study protocols used were the same for all countries and involved three field methods for assessing physical activity (i.e. uni-axial and tri-axial accelerometers and a short non-validated physical activity questionnaire) and five field methods for assessing body composition (i.e. skinfold thickness, circumferences and leg-to-leg bioelectrical impedance) and each compared with respective reference methods (i.e. doubly labelled water, 3- and 4- compartment models, Bammann *et al.*,2010). A brief overview of the study is given in Figure 4. For the purpose of the thesis, the main focus will be on the analysis of the accelerometer data collected during the IDEFICS study only.

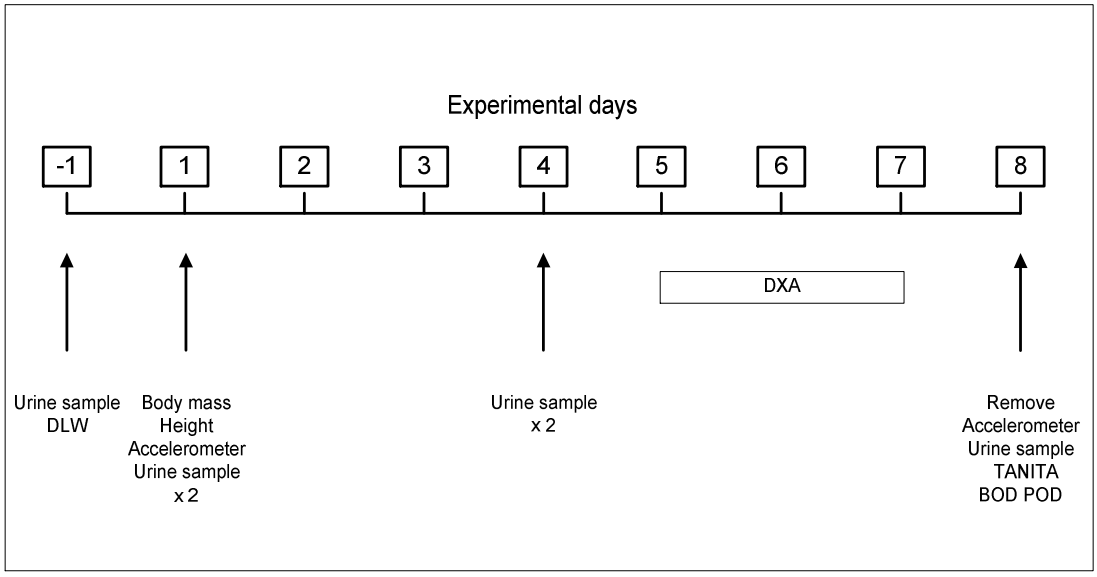


Figure 4: Measurement schedule of the IDEFICS validation study.

The study was conducted over an 8 day monitoring period (Figure 4). In total six urine samples were collected, including a baseline sample taken the night before the study measurement began. Subjects were given an accelerometer to wear on day 1 and were instructed to wear until the final day of measurement, day 8. Between days 1 and 8 anthropometric measures were taken, a dual-energy X-ray absorptiometry (DEXA) scan and body composition measured using a BODPOD® on the final day.

Here the main focus is on the objective assessment of levels of physical activity and sedentary behaviour in the validation study using the uni-axial ActiTrainer accelerometer (Actigraph, LLC, Pensacola, FL, USA); this device was also used to assess physical activity and sedentary behaviour in the IDEFICS study (Ahrens *et al.*,2010). The ActiTrainer device measures accelerations in a vertical axis, within the frequencies of 0.25 and 2.5 Hz. This device uses piezoelectric transducers to convert accelerations into digital signals known as counts. These counts can be summed over a user specified time sampling interval, referred to as epoch and recorded to internal memory. For this validation study, an epoch of 15 seconds was used as previous studies have suggested that an epoch of 1 minute would be too long and might miss the short bouts of high intensity activity typical of young children (Nilsson *et al.*,2006; Reilly *et al.*,2008; Ojiambo *et al.*,2009)

2.2 Measuring Physical activity

Free living sedentary and physical activity times were objectively assessed using the uni-axial Actitrainer accelerometer (Actigraph, LLC, Pensacola, FL, USA). Accelerometers were calibrated before being used using the manufacturer's calibrator (CAL 71, Actigraph, LLC, Fort Walton Beach, Florida). Each subject was given the same model of Actigraph. New accelerometers were bought for this project. Subjects were each given an activity monitor, an accelerometer belt and both parent and subject received an explanation on how to use the device. The activity monitor was worn on the right hip and kept secure against the body at all times using the fitted strap. Parents were also asked to complete a daily diary during the 7-day accelerometer 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 in the morning until it was time for them to go to bed in the evening, so that a full day of physical activity and sedentary behaviour could be

assessed. These times, as well as any other time of the day when the device was removed and reattached, were recorded in the diary. Parents of subjects were instructed to remove the accelerometer during showering or bathing as the device is not waterproof. Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca 225, Seca GmbH & Co. KG., Hamburg, Germany) and body mass was measured using an electronic balance (prototype suitable for measuring leg-to-leg bio impedance in small children based on TANITA BC 420 SMA, TANITA Europe GmbH, Sindelfingen, Germany).

2.3 Accelerometer data analysis and editing

Accelerometer data were analysed using both a standard manual and automated method of analysis in order to assess the performance of the automated method of analysis. The manual process of analysis involved the data being downloaded using ActiLife software (ActiGraph, Pensacola, FL, USA) and all outcome variables computed in Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, USA). This involved pasting the raw data into a spreadsheet and then running a macro, which was written by Dr John Reilly and Victoria Penpraze and referenced in published data (Penpraze *et al.*,2006). This macro calculated counts per minute, sedentary-, light-, moderate-, and MVPA- intensity levels, and other summary statistics for each subject. Data was initially downloaded from the accelerometer onto a computer using the Actilife software version 4.4.1 (Actigraph, LLC, Fort Walton Beach, Florida), which divides activity into separate days running from 00:00 to 23:59. Once each day had been down loaded, the data was then copied to the spreadsheet and the Reilly and Penpraze macro was run. The macro times began at 06:00, and ended at 23:00, if the subject had data that was before or after this time then the macro was edited to suit. Each row on the macro represented 15 minutes, the first row of the day would be 06:00:00 and the next would be 06:15:00. This meant that every 15 minutes of the day had to be copied and pasted into the time row it represented, until the accelerometer no longer showed any counts. The manual method relied heavily on the accelerometer diaries provided to the parents of the subjects to record device ON/OFF times. Accelerometer wear time was input manually by referring to the ON/OFF times reported in the accelerometer diaries. Each day the ON time was input as the start of the third complete minute of counts that were found to be greater than zero after the recorded ON time. Device OFF times were input into the macro as the end of the third minute before the removal of the activity monitor prior to the subject retiring to bed for

evening sleep. The reason for this was that the first and last two minutes of monitoring were counts which may have been produced from moving the device on and off of the subject, and therefore, the removal of these counts would mean that these counts would not be included in the analysis (Penpraze *et al.*,2006). Although this manual method requires diary input which may be seen as making the results less accurate this method was chosen as it combines both subjective and objectives techniques so it would be referred to as a 'Secondary Criterion' (Figure 2). Although the gold standard criterion of DLW for measuring energy expenditure was used in this study, the results have not yet been analysed in time for this thesis write, so using accelerometer data and activity diaries was seen as being close to direct observation as could be for this study and would still produce reliable results.

Inputting and manipulating seven days worth of data from one subject is very time consuming and takes about 2 hours to complete. Once data has been entered and start/stop times have been manipulated for each day, a summary sheet is generated. This sheet includes: total CPM, total monitoring time, number of minutes spent in each physical activity, intensity of each cut-off, and percentage of overall time spent in the specified activity level. The primary difference between manual and automated analysis is how non-wear time is excluded from the data, with the manual method exclusion based on diaries, whereas the automated method used an algorithm developed using R, (version 2.9.0., R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org). A series of computer commands were developed to be used within R which enabled R to automatically read in raw accelerometer files, re-integrate the date to 30 s and 60 s epoch and to exclude invalid data. Excluding data involved the removal of 20 minutes or more of consecutive zeros zero counts prior to further analysis as recommended by Treuth *et al*., (2003) who previously found that a period of 20 min or more of consecutive zero counts was not consistent with the awake state. In order to examine how to define non-wear time, the R programme was also used to remove 10-, 20-, 30-, and 60-minutes of consecutive zeros. The output generated by R included the same summary statistics as in the manual analysis (see Appendix 3 for example of summary sheet). The automatic analysis of all 96 data files with R took less than 5 minutes to compute.

2.4 Data reduction decision rules

Although many studies using accelerometers have been published, there is still not a standardised data reduction method established, including such aspects as defining wearing period of accelerometer in a day, how to measure or estimate ON/OFF wear time during the day and which algorithm to use (Masse *et al.*,2005). Three data reduction decision rules were applied to validation study data to see what effect they had on accelerometer outcome results, these were:

1. Effect of deleting consecutive zeros to define non wear time of the accelerometer: The effect of deleting 10-, 20-, 30-, and 60-minutes of consecutive zeros to define non-wear time was examined on average CPM, sedentary and MVPA behaviour in the EU validation study participants.

2. Effect of epoch: Data were collected using an epoch of 15 seconds. This data was then re-integrated from 15 s, to 30 s, and to 60 s using the specifically designed R programme which summed the activity counts from the required time frame to give out the required epoch output.

3. Effect of cut-point: Data was analysed using previously mentioned published cut-points of Sirard et al. (2005), Pate *et al.*,(2006), Reilly *et al.*,(2003) and Puyau *et al.*,(2002). These cut-points were used to determine how much time was spent engaging in sedentary activities and moderate and vigorous physical activity (MVPA) over the 7 day monitoring period. These cut-points were chosen for the specific age range of the IDEFIC subjects, who were between the age of 4 and 10 years old. Both Pate and Sirard cut-points were created using subjects aged 3-5 years old, Reilly cut-points were created to establish between the inactivity and active of children aged 3-4 years old and Puyau measured children who were aged between 6 and 16. For sedentary activities these were: Sirard: <398 counts·15 s^{-1} and Reilly: <1100 counts·min^{-1} and for MVPA these were Sirard: >890 counts·15 s^{-1}; Pate: >420 counts·15 s^{-1} and Puyau: >3200 counts·min^{-1}. Specific cut-points were either divided up or down, dependent on what epoch the cut points were originally measured with. As Reilly and Puyau's cut points were recorded using a 60 second epoch, the activity counts had to be

divided by two to find 30s epoch effect, and divided by 4 to estimate the 15s epoch results. Pate and Sirard cut points were established using a 15 second recording, therefore, activity counts had to be multiplied up 2 times, and 4 times.

2.5 Reliability of accelerometer variables

Reliability coefficients for accelerometer outcome variables over several days of monitoring for at least 6-hours per day were computed using Intra-class correlation coefficient (ICC), defined as:

$$ICC_S = \sigma_b^2 (\sigma_b^2 / (\sigma_b^2 + \sigma_w^2)$$

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, which uses ICC as a measure of reliability, defined as:

$$N = [ICC_t / 1 - ICC_t] [1 - ICC_s] / ICC_s]$$

Where N is the number of measures or days needed, ICC_t is the desired level of reliability (usually – 0.7, 0.8, or 0.9), and ICCs is the single day reliability. Subjects were excluded if they did not have more than 6 days of data with at least 1 weekend day and 6 hours or more of physical activity data per day.

2.6 Data analysis

In order to compare the two measurement techniques, the Bland & Altman (Bland and Altman, 1986) method was used. An independent t-test was also performed to test the difference between the two methods (MedCalc version 8.0.0.0; http://www.medcalc.be). Data were expressed as mean (standard deviation (SD) or median (range) following Kolmorgorov-Smirnov 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 repeated measures, ANOVA followed by Bonferroni *post-hoc* test. 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 15.0 (SPSS, inc., Chicago, IL).

3. Results

3.1 Physical Characteristics of subjects

Of the total sample of 98 children recruited by the four different centres, 86 fulfilled the inclusion criteria for data analysis (at least 6 days including at least 1 weekend day of valid recording of at least 360 min of continuous monitoring per day). The individual subject characteristics of these 86 subjects are given in Table 2, and further displayed in Figures 5-8. Children from Gothenburg were included into the study as the number of obese children required for the study had not yet been met, so it was suggested to use the children already attending the obese clinic in Sweden. This brought the figure over 30% of the subjects (17) being either overweight or obese, which was more in line with the study group spread required. As mentioned previously, the purpose of this study is to analyse accelerometer data. It did not matter that the Gothenburg subjects were overweight/obese as there was still data to download and compare between manual and automated methods. Figure 6 shows the difference in weight of each subject, between centres. The weight difference can be seen quite clearly in Figure 6, with Gothenburg having much heavier subjects. The initial age range requested was between 4-8 years old, however both Glasgow and Gothenburg found it difficult to recruit children of this age and the age was increased to 4-10 years old as a result (Figure 7). Figure 8 displays the average CPM of each subject, with each centre being compared. The box plot shows the wide spread of CPM in each centre, however a T-test found there to be no significant difference between the centres ($p=<0.0001$). The lowest CPW was found in the Gothenburg centre, as low as 247.3 CPM by a subject. This same subject as also the heaviest subject included in the study at 61kg (Table 2), which does back up the relationship between low activity levels and obesity in children previously mentioned in this these. Subjects were monitored using uniaxial accelerometry for a daily average of 11.7 ± 1.7 hours (Table 4). During the monitoring period, a high compliance to wearing the units throughout the day was essential in order to successfully validate the automated method of analysis of accelerometer data and to assess the habitual behaviour of each subject. Table 3 describes subject adherence to wearing the accelerometer device for more than 6 hours each day. Subject adherence remained above 90% for the first 6 days of the week-long study period. 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 measurements (see Bammann *et al*, 2010) and had to remove the accelerometer. If the final day data from UZAZ are removed from the adherence analysis, compliance on the final day remained high at 75%.

3.2 Manual vs. automated results

There was excellent agreement in accelerometer outcome variables between the manual vs. automated analysis (Table 4). The daily average time spent sedentary for both methods and cut-offs are shown in Table 4 and respective Bland-Altman plots illustrated in Figures 5 for Sirard and Figure 6 for Reilly sedentary. Sirard and Reilly both had small bias and narrow limits of agreement when comparing manual vs. automated, shown in Figures 5 and 6, respectively. The average difference between methods in these Bland-Altman plots for Sirard was 10.9 minutes and 7.1 minutes for Reilly (Figure 6.7). On an average day, the automated (A) analysis underestimated the sedentary behaviour by less than 16 minutes compared to the results of the manual (M) analysis when using Sirard (A, 586 ± 83 vs. M, 602 ± 77; P>0.05) and 13 minutes when using Reilly cut-points (A, 555 ± 77 vs. M, 568 ± 75; P>0.05) (Table 4, Figures 5 and 6, respectively). An independent t-test found that there was also no significant difference in the time spent in MVPA in each cut-point, regardless of whether the analysis was conducted manually or automated (Sirard A, 18 ± 10 vs. M, 19 ± 10; Pate A, 78 ± 22 vs. M, 75 ± 22; and Puyau M, 13 ± 10 vs. A, 15 ± 10) (Table 4, Figures 7, 8, and 9 respectively). When comparing manual vs. automated analysis, Sirard, Pate and Puyau cut points again showed narrow limits of agreement and small bias (Figures 7, 8 and 9 respectively). The average differences between the two methods were all very low, Sirard 0.2 minutes, Puyau 0.0001 minutes and Pate 0.63 minutes. The Pate cut points showed slightly wider limits of agreement, but with the average difference being less than 60 seconds, the agreement between the two methods is high (Figure 8).

3.3 Reliability of accelerometer variables

Table 5 shows the reliability coefficients of accelerometer variables over the monitoring period. Single day ICC for average CPM was 0.32 for CPM, 0.33 for sedentary time and 0.35

for MVPA. The number of days (including at least 1 weekend day) required to obtain 80% reliability for average CPM, sedentary and MVPA was 8.5, 8.1 and 7.4 days, respectively.

3.4 Data reduction analysis

***Sedentary time**:* Choice of epoch and cut points had a significant effect on sedentary time ($P<0.001$). *Post hoc* analysis revealed significantly less sedentary time per day when using Reilly cut points 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, respectively (Table 6). In contrast Puyau cut-points 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 6). There was no significant ($P=0.007$) difference for Sirard cut-points across all the 3 epochs evaluated. However, Sirard cut-points reported significantly ($P<0.001$) more sedentary time compared to Reilly and Puyau cut-points using 15 s, 30s and 60s epoch (Table 6).

Moderate to vigorous physical activity (MVPA): Choice of epoch and cut-points also had a significant effect on MVPA time (Kruskal-Wallis, $P<0.001$). Mann-Whitney U analysis revealed significantly more MVPA time using Pate, Sirard and Puyau cut-points when comparing 15 s vs. 30 s and 15 s vs. 60 s epochs (Table 6). When comparing different cut-points, the Pate cut-points reported significantly more MVPA time compared to Sirard and Puyau cut-points ($P<0.001$) across all the epochs (Table 6).

Determining non wear time by the removal of consecutive zeros: There was a significant difference in time spent being sedentary when comparing 10 minutes vs. 20 minutes, 10 minutes vs. 30 minutes, and 10 minutes vs. 60 minutes (Table 7). The largest difference was found in sedentary behaviour between the removal of 10 minutes of consecutive zeros and 60 minutes (572 ± 67 min vs. 628 ± 67 min**;** $P<0.01$) (Table 7). There was no significant difference found between CPM or MVPA, regardless of how many minutes of consecutive zeros were removed (Table 7).

3.5 BMI z-scores

There was found to be no relationship between physical activity, sedentary behaviour and BMI z-scores of the IDEFICS subjects used in this current study. Figure 10 shows that there was no correlation between BMI z-scores and sedentary behaviour ($r = 0.16$, $p = 0.89$). This same figure also shows that percentage time in MVPA and average CPM showed no correlation with BMI z-scores ($r = -0.13$, $p = 0.26$ and $r = -0.01$, $p = 0.26$, respectively). It was decided to remove the Gothenburg subjects from this analysis as all of their subjects were taken from an obese clinic in Gothenburg and this may have been affecting the BMI z-score analysis. However, removing them from the analysis did not change the output of the relationship of BMI z-scores with physical activity and sedentary behaviour (Figure 11).

Table 2: Descriptive characteristics of included children in each validation centre.

Centre	ID	Sex	Height (cm)	Weight (kg)	Age (years)	CPM (minutes)
UGHENT	IV01	M	127.1	23.6	8	574.2
	IV02	F	117.9	22.3	6	512.1
	IV03	F	103.8	20.5	4	549.0
	IV04	M	127.7	23.5	7	593.9
	IV05	F	106.9	16.6	6	499.4
	IV08	F	107.6	17.4	4	572.8
	IV09	M	126.3	25.8	7	611.6
	IV10	F	114.3	22.6	5	681.6
	IV11	M	117.6	22.5	5	762.1
	IV12	M	128.2	29.1	7	541.8
	IV13	F	100.5	13.9	4	592.2
	IV14	M	126.7	25.7	7	476.2
	IV15	F	116.5	21.6	7	879.8
	IV17	F	119.5	23.6	6	434.5
	IV18	F	102.6	15.8	4	482.1
	IV20	F	125.2	23.5	7	793.2
	IV21	M	105.3	17.3	4	666.3
	IV22	F	104.0	16.2	4	549.1
	IV23	F	128.4	26.3	6	503.8
	IV24	M	137.6	26.6	8	572.1
	IV25	F	125.4	27.2	6	602.4
	IV26	F	136.9	28.3	8	620.6
	IV27	F	115.7	20.1	5	633.0
	IV28	F	119.0	21.8	5	551.6
	IV29	M	126.0	23.1	7	585.6
	IV30	F	127.0	25.1	8	580.6
	IV32	F	109.9	21.4	4	670.3
	IV33	F	113.3	21.1	6	650.1
	IV34	F	106.0	14.7	4	514.9
	IV35	M	139.7	43.4	8	587.6
	IV36	M	109.1	20.3	4	555.1
	IV37	F	128.6	24.7	7	599.3
	IV38	M	112.0	17.4	4	638.9
	IV39	F	123.3	25.1	5	696.3
	IV40	M	134.4	29.6	6	576.2
UGLW	IV01	M	125.5	25.5	6	587.7
	IV02	M	111.6	18.5	5	488.2
	IV03	F	102.2	18.7	4	381.2
	IV04	M	131.0	25.5	8	581.6
	IV05	M	137.7	31.0	10	680.2
	IV07	M	123.2	21.7	8	505.8
	IV08	M	127.8	28.4	7	621.6
	IV09	M	132.6	26.8	8	527.9

	IV10	F	119.9	22.8	7	573.6
	IV11	F	113.0	19.4	7	727.7
	IV12	F	128.5	29.5	7	698.8
	IV13	M	130.0	33.1	7	598.1
	IV14	M	124.4	24.8	7	764.4
	IV16	F	135.0	34.6	9	499.9
	IV17	F	136.3	38.4	9	440.9
UGOT	IV00	M	120.0	35.6	5	597.9
	IV01	M	131.0	43.8	6	715.1
	IV04	F	115.0	29.8	4	498.1
	IV05	M	114.0	26.9	4	948.6
	IV07	M	132.0	41.6	7	706.5
	IV08	F	140.0	48.0	9	522.8
	IV09	M	160.0	61.0	10	247.3
UZAZ	IV02	F	125.4	24.7	7	399.6
	IV03	F	116.7	35.4	8	375.9
	IV05	F	116.2	19.7	5	734.1
	IV06	F	127.6	29.7	7	354.3
	IV08	F	121.9	24.3	6	329.8
	IV09	F	110.6	18.2	5	625.0
	IV10	M	121.8	20.3	8	499.5
	IV12	F	116.0	20.2	5	409.7
	IV14	M	120.8	25.7	6	513.6
	IV16	M	116.4	19.4	7	618.2
	IV18	M	118.0	23.1	5	664.5
	IV19	M	113.8	22.1	4	578.7
	IV20	M	132.0	24.0	8	689.6
	IV21	M	111.5	19.4	5	651.8
	IV22	F	126.0	25.1	8	500.0
	IV23	M	110.0	18.7	5	561.2
	IV24	F	125.5	29.5	8	434.5
	IV25	F	113.8	22.3	5	636.1
	IV27	F	120.4	23.7	5	355.5
	IV28	M	127.0	25.8	8	613.9
	IV30	M	135.9	30.0	7	666.6
	IV31	M	126.4	22.2	8	667.1
	IV33	F	118.0	20.9	5	309.5
	IV36	M	112.2	19.7	6	689.4
	IV38	M	114.8	19.7	5	838.2
	IV39	F	120.6	30.3	6	541.2
	IV40	F	129.9	28.6	9	503.2

Table 3: Number of valid files (≥6 hours of data) during the 7 day monitoring period.

	1 Day	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days
UGHENT	38	35	35	35	35	34	29
UGLW	16	16	16	15	15	15	13
UGOT	10	9	7	7	7	7	7
UZAZ	32	32	32	30	30	30	1
TOTAL	96	92	90	87	87	86	50
% valid	100	96	94	91	91	90	52

Table 4: Table describes average daily time spent in each activity cut-offs, either whilst sedentary or in MVPA.

	Time spent in Sedentary Activity (minutes)		Time spent in MVPA (minutes)		
Analysis	Sirard	Reilly	Sirard	Puyau	Pate
Manual Mean (SD)	602 (77)	568 (75)	19 (10)	15 (10)	75 (22)
Automated Mean (SD)	586 (83)	555 (77)	19 (10)	16 (10)	76 (22)

Table 5: Reliability of accelerometer outcome variables over several days of measurement

		Days of Measurement[b]		
Parameter	ICC[a]	R = .7	R = .8	R= .9
CPM	0.32	5	8.5	19
Sedentary	0.33	4.7	8.1	18.3
MVPA	0.35	4.3	7.4	16.7

ICC Intra-class correlation coefficient (intra-individual/total variation).
a Based on 6 days (defined here as ≥ 6-hr of monitoring) of monitoring including at least 1 weekend day.
b Predicted by Spearman-Brown Prophecy formula.

Table 6: Time in minutes in sedentary and MVPA across all epochs as determined using Reilly, Puyau, Sirard and Pate cut-points. Data presented as mean (SD) for sedentary time and median (range) for MVPA.

Sedentary	15	30	60
Sirard	616±94[c,g]	620±95[c,g]	624±97[c,g]
Puyau	548±90[g]	541±91[a,g]	536±92[a,g]
Reilly	570±91	579±93[a]	579±94[a]
MVPA	**15**	**30**	**60**
Sirard	18 (1-80) [b,d,e,f]	12 (0-70) [a,d,e,f]	9 (0-71) [a,b,d,e,f]
Puyau	24 (1-100) [b,d,f]	18 (0-93) [a,d,f]	13 (0-84) [a,b,d,f]
Pate	78 (4-197) [b,d,e]	72 (3-202) [a,d,e]	66 (1-201) [a,b,d,e]

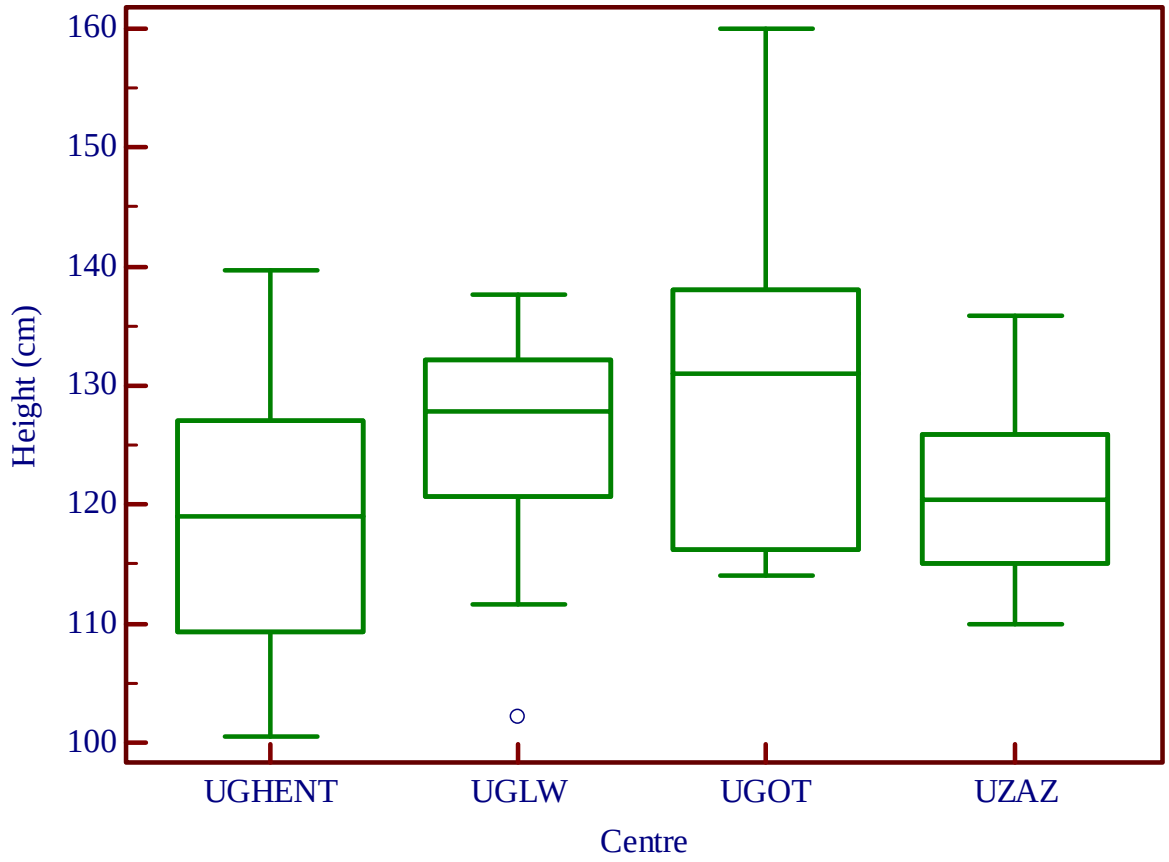
a and b: indicate significant difference from 15 s and 30 s epoch, respectively.
c: indicates significant difference between Reilly vs. Sirard cut-points.
d: indicates significant difference between Sirard vs. Puyau and Pate cut-points.
e: indicates significant difference between Pate vs. Sirard and Puyau cut-points.
f: indicates significant difference between Puyau vs. Sirard and Pate cut-points.
g: indicates significant difference between Sirad vs. Puyau and Reilly cut-points.

Table 7: Definition of non-wear time as 10, 20, 30 and 60 min of consecutive zeroes on physical activity parameters, epoch setting 15 s (values are means ± SD).

Parameters	10 min (N=86)	20 min (N=86)	30 min (N=86)	60 min (N=86)
CPM	593 ± 127	570 ± 125	562 ± 123	543 ± 120
Sedentary (min)	572 ± 67*	597 ± 67†*	607 ± 68†*	628 ± 67†
MVPA (min)	20 ± 9	20 ±9	20 ± 9	20 ± 9

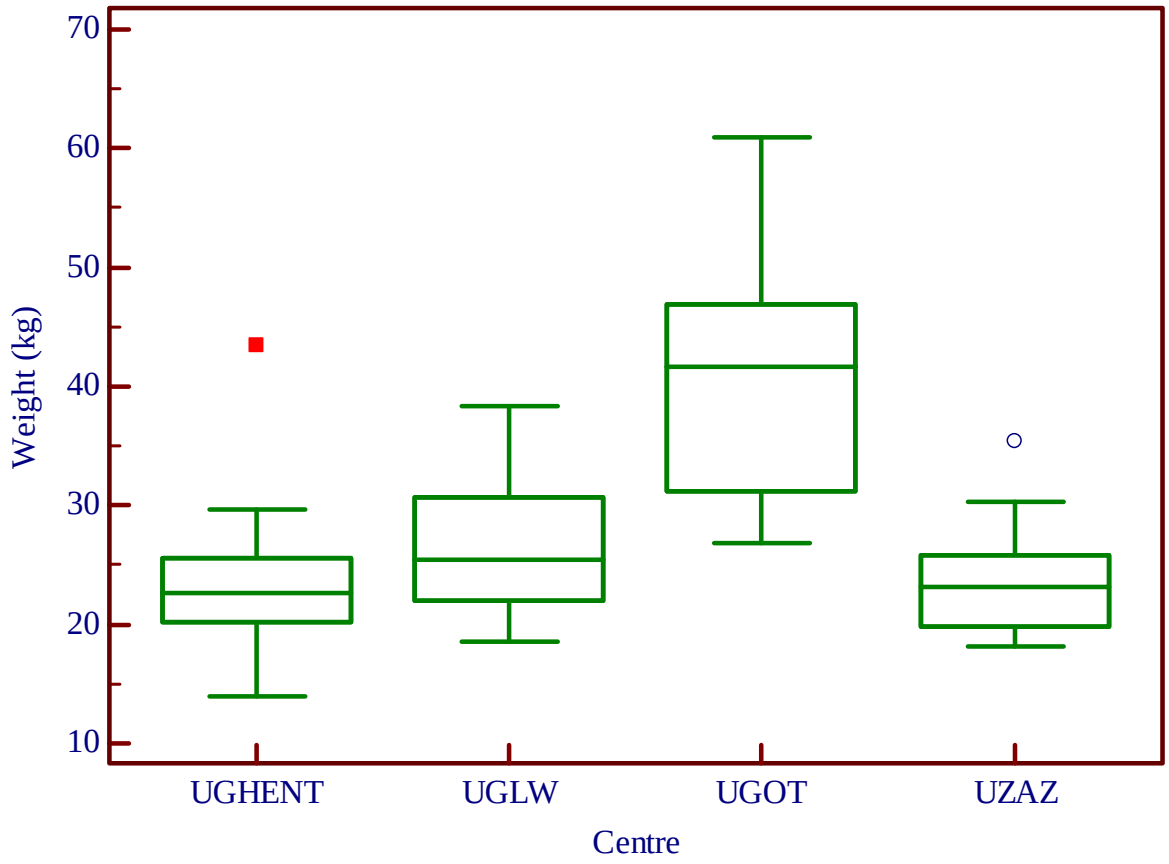
N-Subject numbers
CPM - Counts per minute
MVPA- Moderate to vigorous intensity activity
† - Significant difference from removal of 10 minutes of consecutive zeros
* - Significant difference from removal of 60 minutes of consecutive zeros

Figure 5: Box plot comparing the height of subjects in each validation centre



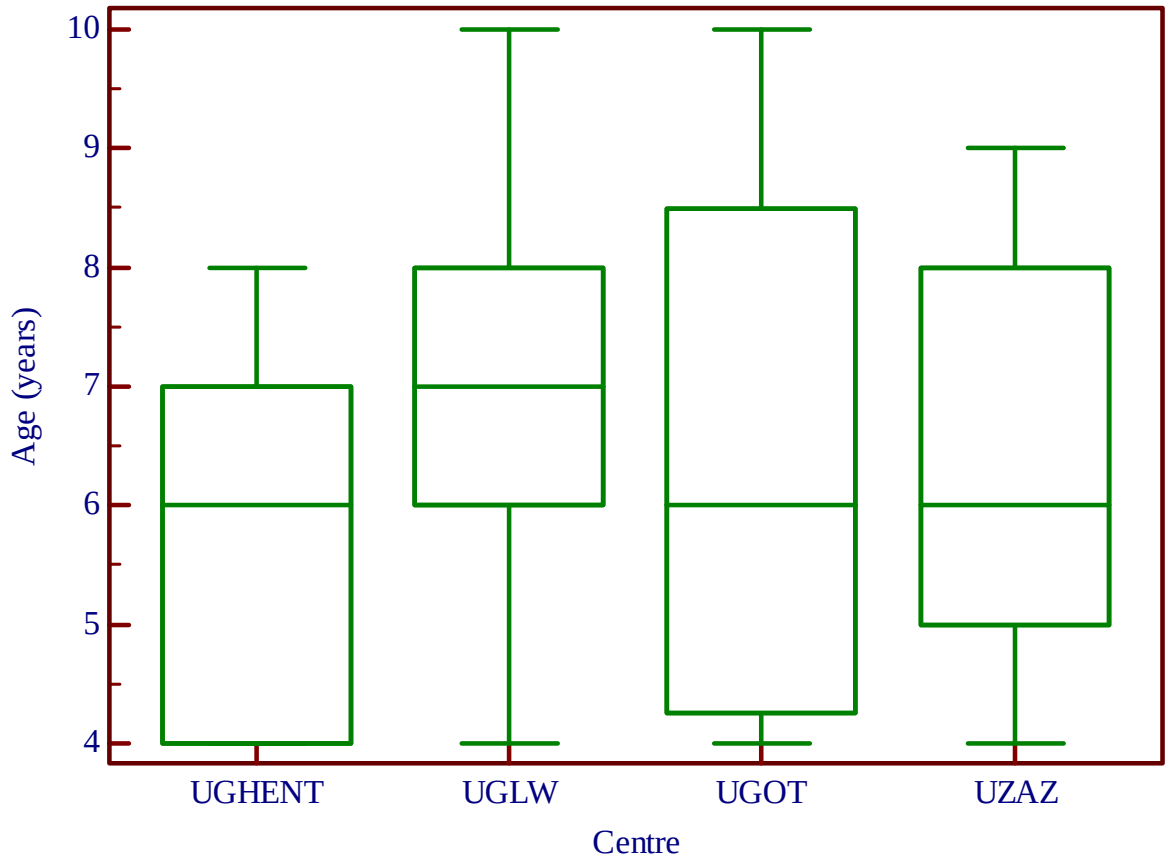
The box plot displays the height of subjects from each centre. A one way Anova test showed that the children from the University of Gothenburg were significantly taller (p=0.02). There were no significant differences between the other three groups (p=0.12).

Figure 6: Box plot comparing the weight of subjects in each validation centre



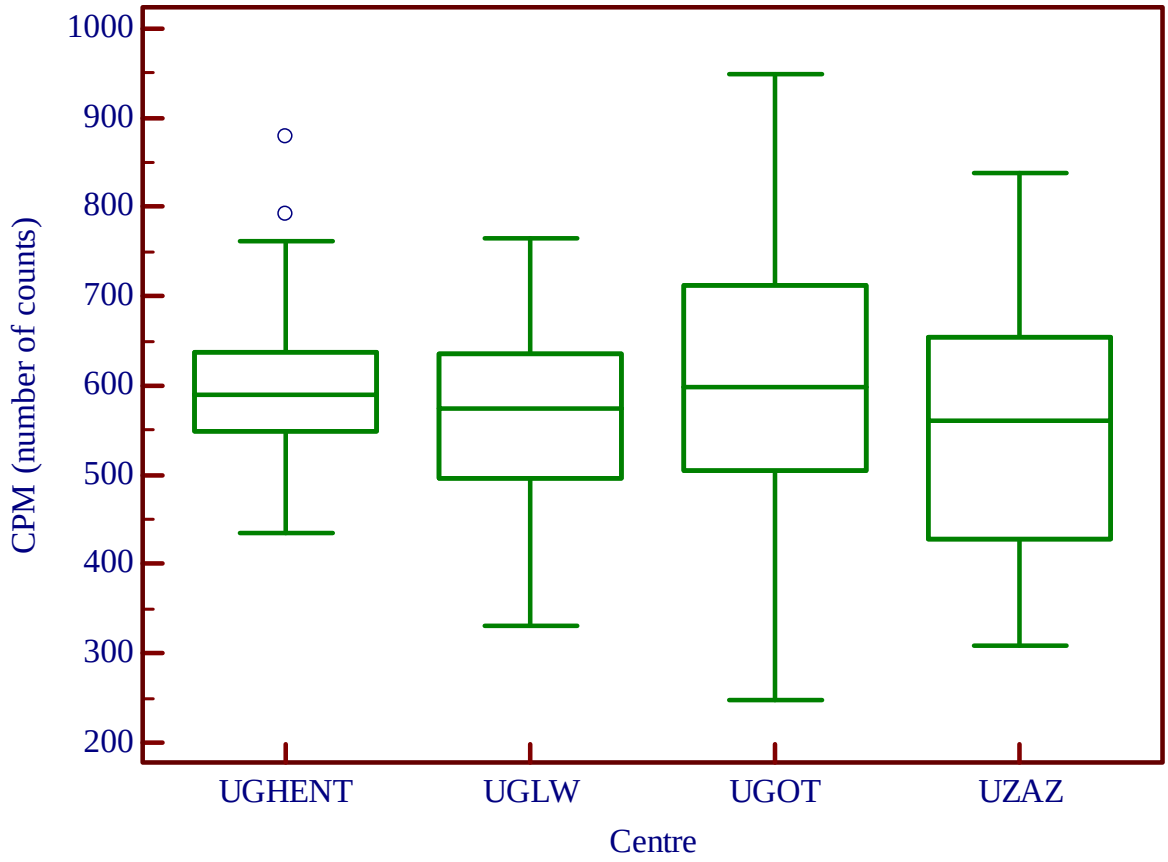
The box plot displays the weight of subjects from each centre. A one way Anova test showed that the children from the University of Gothenburg were significantly heavier (p=0.001). There were no significant differences between the other three groups (p=0.069).

Figure 7: Box plot comparing the age of subjects in each validation centre



The box plot displays the age of subjects from each centre. A one way Anova test showed that the children from the University of Glasgow were significantly older (p=0.027). There were no significant differences between the other three groups (p=0.333).

Figure 8: Box plot comparing the number of CPM used subjects in each validation centre



The box plot displays the CPM of subjects from each centre. A one way Anova test showed that there were no significant differences between the groups (p=0.361).

Figure 9: Time spent in sedentary activity, comparing manual method with automated analysis using Sirard cut points

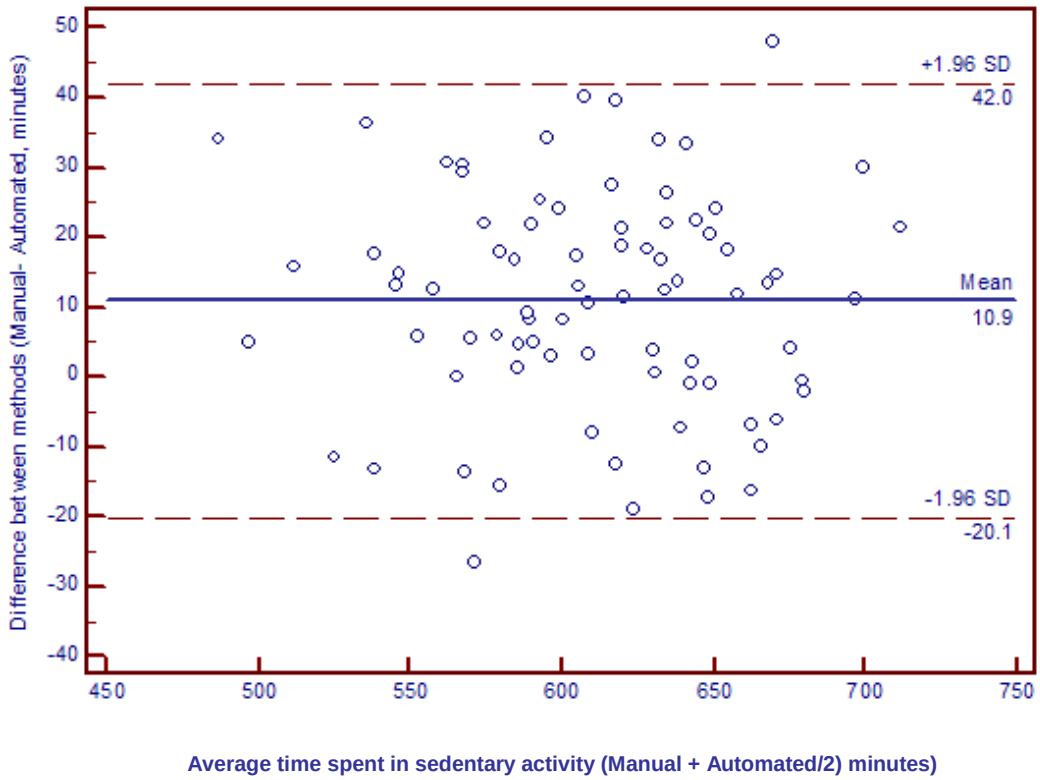


Figure 10: Time spent in sedentary activity, comparing manual method with automated analysis using Reilly cut points

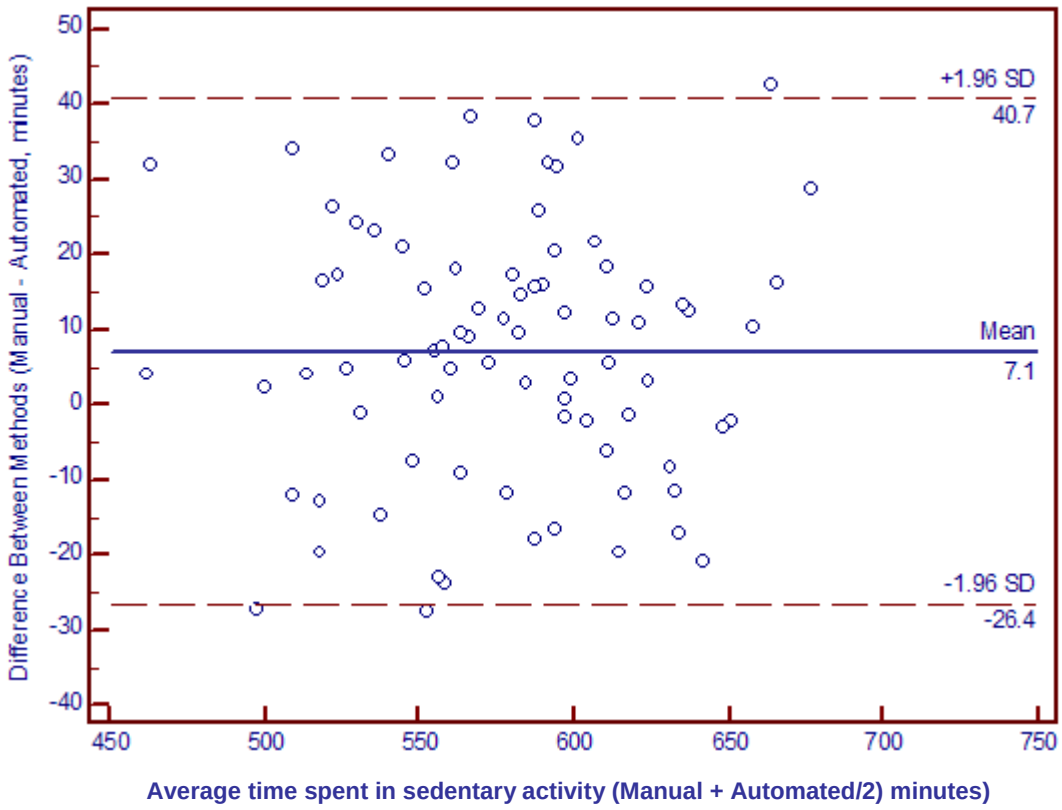


Figure 11: Time spent in MVPA comparing manual method with automated analysis using Sirard cut points

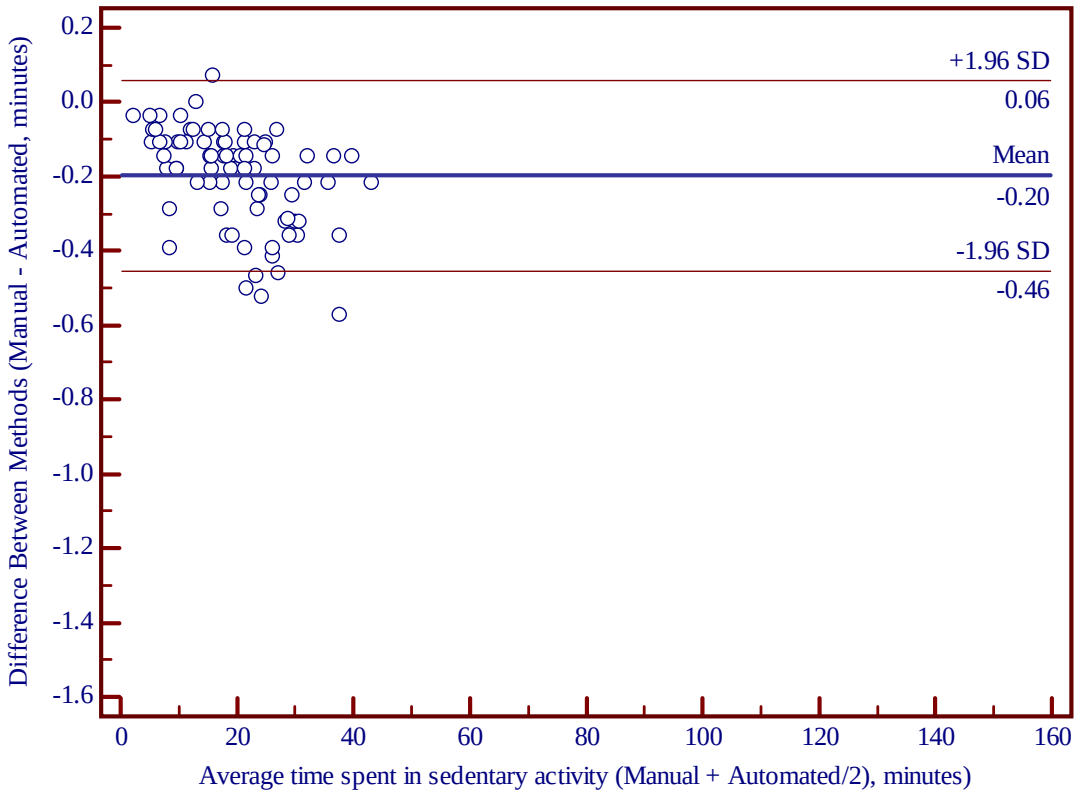


Figure 12: Time spent in MVPA comparing manual method with automated analysis using Pate cut points

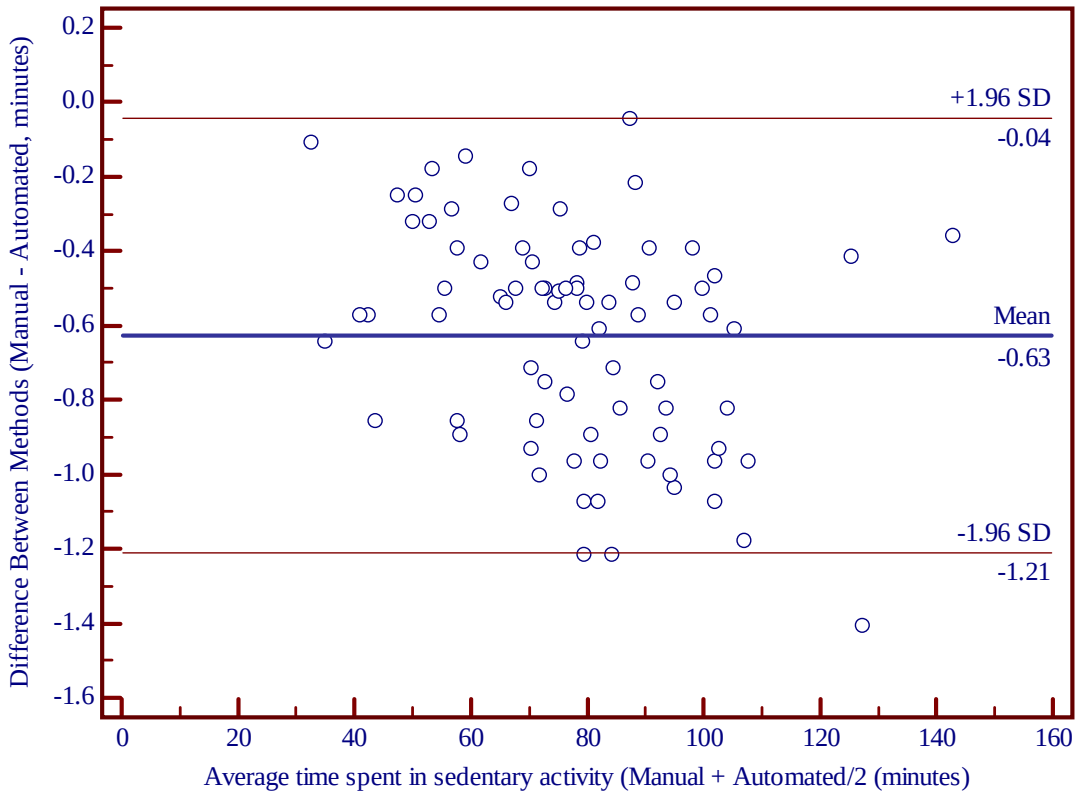
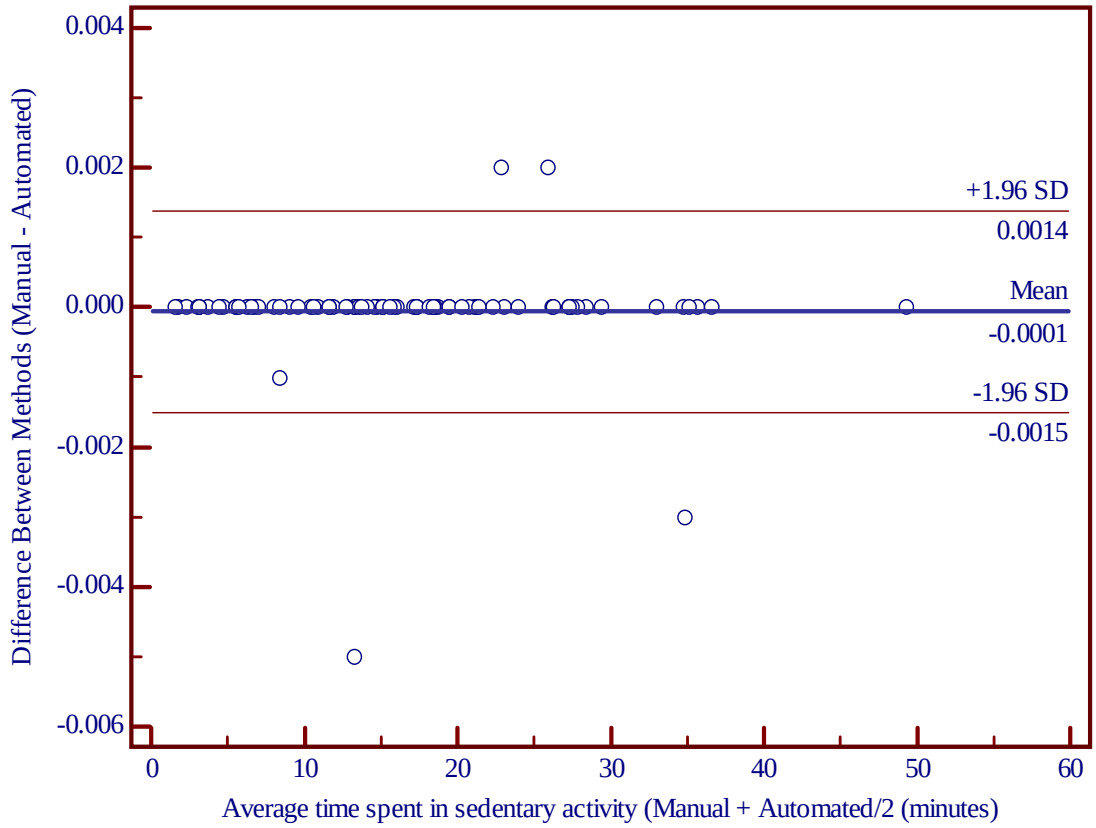
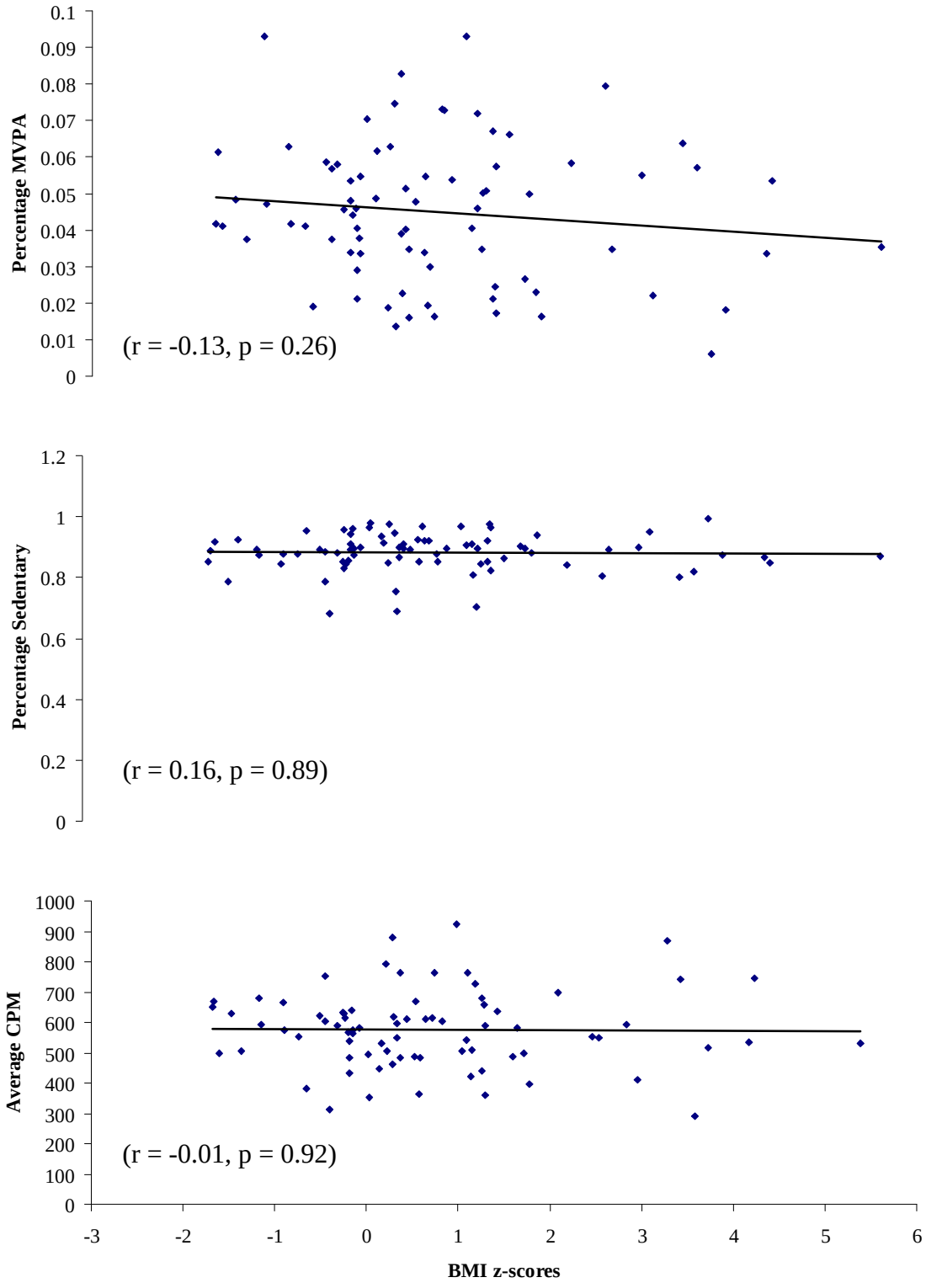


Figure 13: Time spent in MVPA comparing manual method with automated analysis using Payau cut points



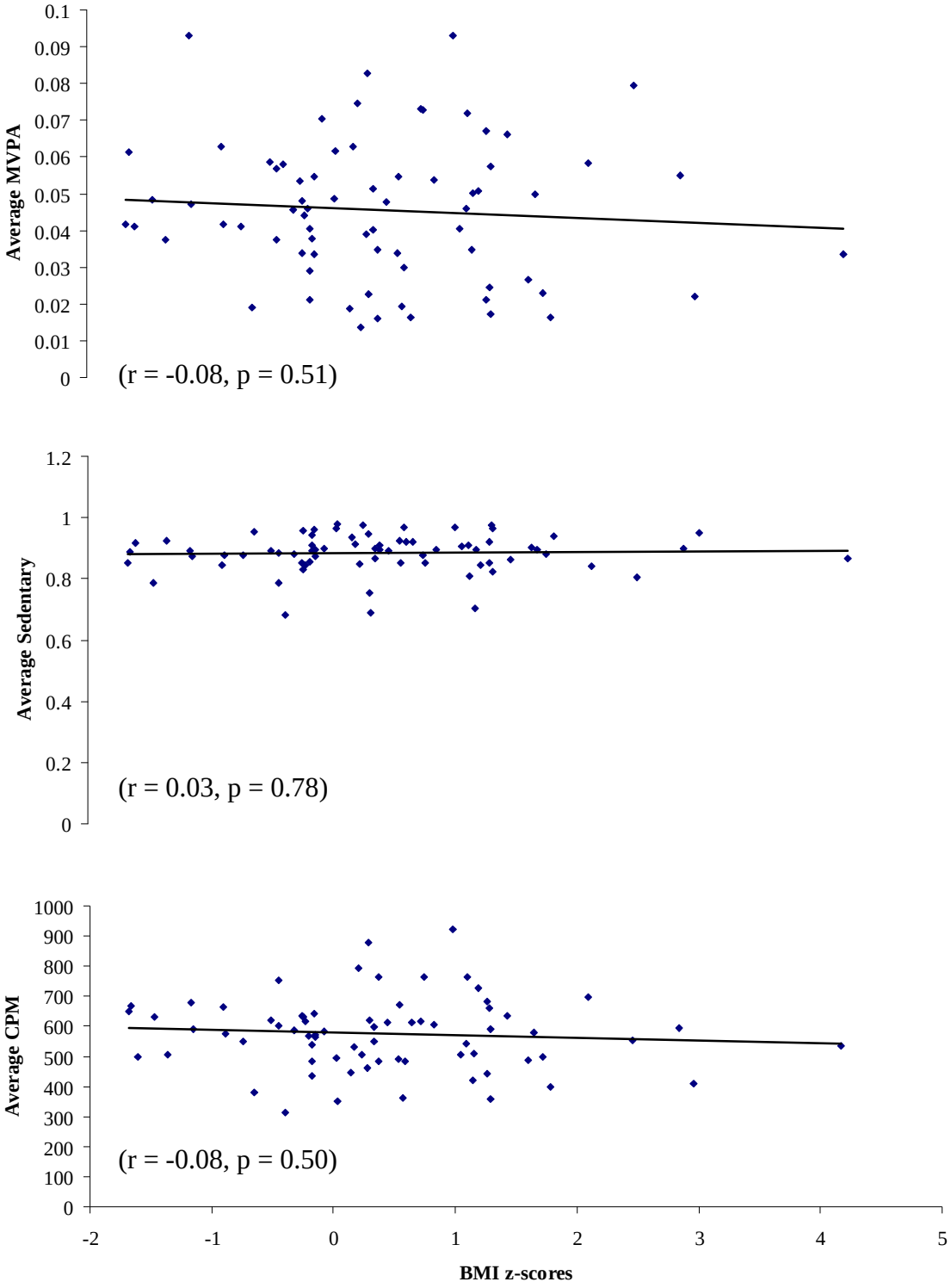
Figures 9-13: Biases (mean errors, time in minutes, solid horizontal line) and limits of agreement (1.96 X SD of the errors, broken horizontal line) for the three cut-points, manual plotted against automated using Bland-Altman.

Figure 14: Z-scores, using all Validations centres



Graphs show that BMI z-scores were not affected regardless of CPM, time spent in sedentary or MVPA.

Figure 15*:* Z-scores, not including Gothenburg



By removing Gothenburg the BMI z-scores remained unchanged.

4. Discussion

4.1 Comparing standard manual with automated accelerometer analysis

As part of the IDEFICS Validation study, one aim of this study was to describe and validate an automated method of analysis of accelerometer data for use in large scale epidemiological studies. Although the difference between sedentary times is not significant, the slight differences in sedentary times between the diary based method and the algorithmic method seen (Table 3) may be due to data reduction. The algorithm used for the automated analysis removed 20 minutes or more of consecutive zeros, as this was considered to be non wear time. The data that was then removed using this algorithm may actually have been true sedentary activity; the subject could have been sat watching TV, resulting in zero counts. The two points on both the Sirard and Reilly Bland Altman graphs (Figures 5 and 6, respectively) are of the same two subjects, it could be that the parent of the child has not completed the activity diaries correctly and may not have recorded the correct ON/OFF times. These two subjects were both from the Gothenburg cohort, where it was mentioned during collection that the weather had been particularly hot so the children had been taking off the accelerometers themselves and then putting back on later – this may have happened with these two children and their parents were unaware that the accelerometer had been removed. However, there is also the possibility that the parents forgot to record some of the non-wear periods, making the diaries inaccurate but without directly observing their behaviour it is difficult to know the reason. The MVPA Bland Altman plots show how closely the two methods compared when measuring this intensity of exercise. Unlike sedentary activity, MVPA would not affected by the removal of 20 minutes of consecutive zeros issue. It is unlikely that long period of inactivity would occur before MVPA occurring and with CPM needing to be high enough to be represented in MVPA it is not a reasonable assumption. Both methods of analysis used the same cut-points, and there would be no removal of zeros during this high energy activity, so ideally results should all be similar, which Figures 7 - 9 clearly show. It is also important to mention that even though some points do lie out with the confidence intervals, even the largest differences between the two models are so small (Sirard's 0.6 minutes, Payau 0.006 minutes and Pate 1.4 minutes, Figure 6.8) that the success of automated analysis can be seen quite clearly. With current physical activity guidelines using MVPA as their activity criteria, it is perhaps more important that manual and automated output agree so significantly.

Another important factor that should be mentioned in the manual and automated analysis is the time cost of each. With manual analysis there is the possibility of human error and the analysis is much more laborious than automated analysis. To manually input the data, one day at a time, requires complete attention to avoid errors, and you are required to rely on the accuracy of the subjective measures (accelerometer diaries). The manual analysis for this study took weeks to complete, whereas using the R programme to complete the automated analysis, the results were generated in a matter of minutes. In fact, it took longer to analyse one subject manually than it did to measure all 96 subjects using R. The algorithmic analysis removes the human error and is clearly capable of analysing huge numbers of data in a matter of minutes compared to manual analysis of accelerometer data files. Although there are a number of accelerometer programmes currently available (MAHUffe, Kinesoft, MeterPlus) to analyse accelerometer files, the R programme has many more beneficial features than the current programmes. Unlike the previously mentioned accelerometer analysis programmes, R can analyse many files at one time – with well over 1,000 files able to be analysed at the one time. The R programme also contains statistical packages, whose analysis can be also be applied to the large number of batches accelerometer files being analysed. This automated method of analysis of accelerometer data has been successfully validated and its use recommended for large scale epidemiological studies.

4.2 Effect of methodical decisions on accelerometer output

This study illustrates the significant effect methodological decisions have on accelerometer outcome variables for physical activity and sedentary time in young children. Choice of epoch had a significant effect on the time spent in sedentary activity (Table 6). Using a 15 s epoch reported significantly lower sedentary time compared to 60 s epochs using Sirard and Reilly cut points (Table 6). Puyau cut points, however, showed the opposite trend with 15 s epoch reporting significantly higher sedentary time compared to a 60 s epoch (Table 6). These findings contradict previous studies which have found no significant difference in time spent in this low activity threshold (Rowlands *et al.*,2006; Reilly *et al.*,2008). This contradictory finding could be explained by the present study having higher subject numbers compared to these previous studies (87 vs. 25 and 32 subjects, respectively). The subjects in this study were fairly inactive, with an average of just under 10 hours of sedentary activity a day (Table 6),

and with average monitoring time being just over 11.5 hours (Table 2), more than 83% of the monitoring time was spent being sedentary. This large time spent in sedentary behaviour suggests that children have similar levels of activity as adults, with only small periods of physical activity occurring, which has been suggested by previous studies (Reilly *et al.*,2008; Cardon and Bourdeauhuif, 2007). If this is the case, it is not surprising that the numbers of children being overweight or obese are on the increase.

Epoch selection had a significant influence on time spent in MVPA, across all three cut points. The trend was to report approximately 10 minutes more in MVPA time with 15 s epoch compared to 60 s epoch (Table 6). Previous studies have also shown that as epoch setting increased, the number of minutes recorded in high intensity activities decreased (Rowlands *et al.*,2006; Nilsson *et al.*,2002). Cut point selection also had a significant effect on reported MVPA time (Table 6). Using Pate cut points, the time spent in MVPA was highest and implied that the subjects were very active, however, Sirard and Puyau cut points suggested much lower MVPA patterns (Table 6). The increased time in MVPA when using Pate cut points is probably the result of the lower cut point threshold (>420 counts·15 s^{-1}) compared to that of Sirard and Puyau (>890 counts·15 s^{-1} and >3200 counts·min^{-1}, respectively). However, this epoch effect was not seen in a recent study (Edwardson and Gorely, 2010) which found that a shorter epoch was actually associated with fewer minutes being measured in MVPA, contradicting the current study's finding. Again, these differences in findings might be due to the large difference in subject numbers. Moreover, these inconsistencies emphasise the need to standardise accelerometer data reduction methods, particularly the effect of a different choice of epoch or cut point can have on physical activity outcome figures.

The percentage of children in the present study meeting the current physical activity guidelines for children of at least 60 minutes of MVPA per day was also significantly influenced by the choice of epoch and cut point. With the large periods of time spent MVPA measured by Pate cut points, about three quarters of the subjects (64 subjects) met the guidelines. This data has not been included as there was 0% compliance to guidelines when using Sirard and Puyau cut points, therefore no comparison can be made between effect of epoch or cut points on subject compliance. Such low adherence to PA guidelines has also been shown in previous studies, with Reilly *et al.*,(2008) and Trost *et al.*,(2007) finding that

Western children are spending as little as 18-20 minutes per day partaking in MVPA, which is much below the guidelines.

In summary, this study has clearly demonstrated the effect that both epoch and cut-points have on sedentary and MVPA classification in young children. Even though it is clear from the results presented that cut-points 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 cut-points has yet to be determined (Roberts and 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, with studies suggesting that longer epoch results mask the moderate to vigorous activity (Cavill *et al.*,2001). The biological significance of the observed differences remains to be determined and is currently being investigated in the IDEFICS study using the doubly labelled water criterion measure to assess energy expenditure in combination with accelerometer outputs (see Bammann *et al.*,2010).

Despite many studies using accelerometers have being published there is still not a standardised data reduction method established, not only identifying minimal wear requirement for a valid day or how to compute outcome variables of the accelerometer but also how to define non wear time of accelerometers (Masse *et al.*,2005). Recent studies have used a range of values of continuous zero counts to identify non wear times in children, these include 10 min (Brage and Wederkopp, 2004; Ekelund *et al.*,2004), 20 min (Treuth *et al.*,2004; Treuth *et al.*,2003), 30 min (Cradoch *et al.*,2004) and 60 min (Masse *et al.*,2005). In this present study, the effect of identifying whether long continuous bouts of accelerometer inactivity are due to the accelerometer being removed or actually that the child has been completely inactive during that time is very difficult to measure. Continuous zeros can results from sitting still for long periods of time, removal of device during water activities (showering, swimming or being physically active) or even simple malfunction. Using these different decision criteria affects many different outcome variables when assessing physical activity (Masse *et al.*,2005). This same study is the first of its kind to demonstrate the importance of having a standard accelerometer data reduction, as outcomes of their study using 60 min and 20 min of consecutive zeros as non-wear time produced differing levels of

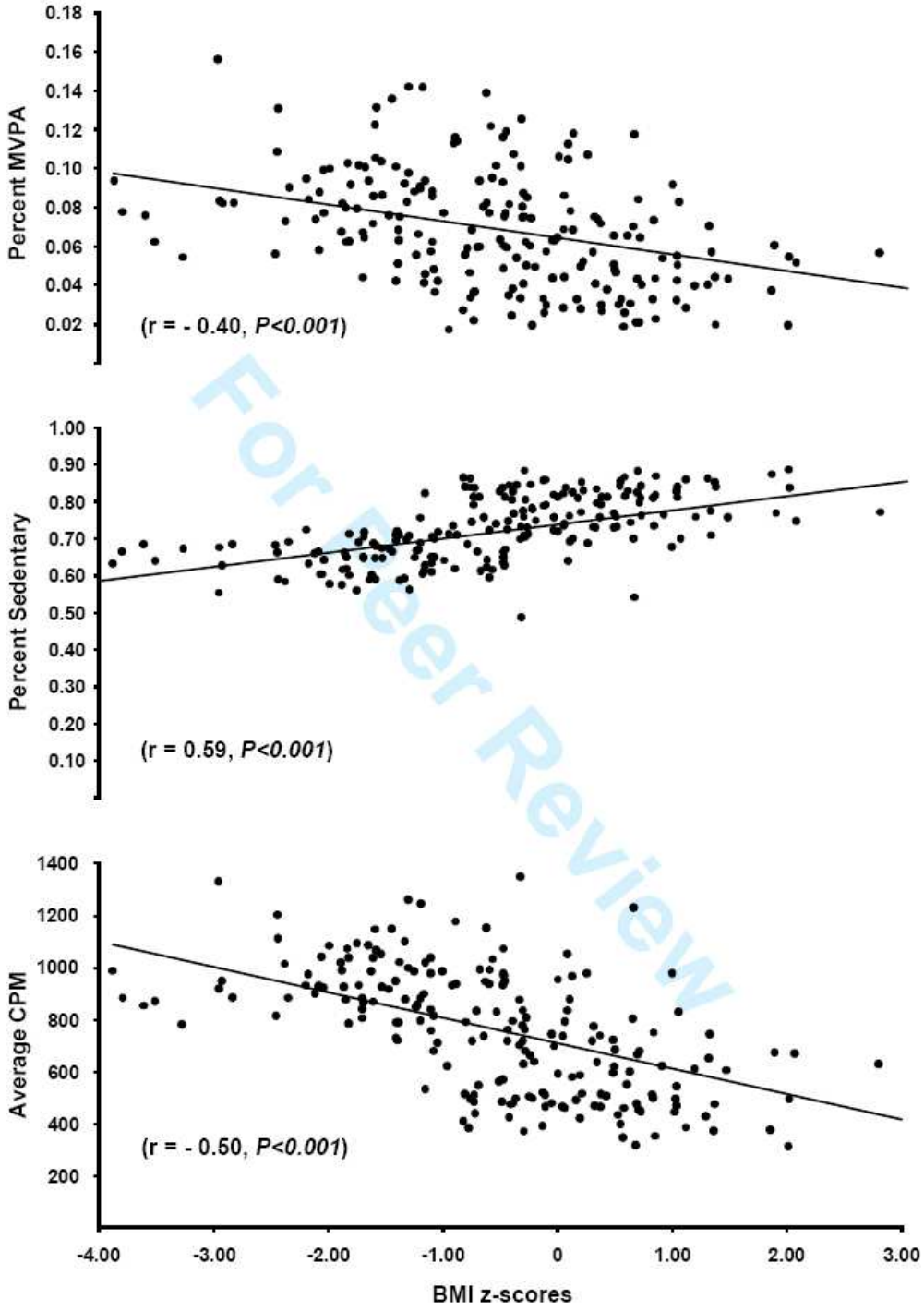
physical activity output. It is difficult to decide how many consecutive zeros represent non-wear time in children.

4.3 BMI Z-scores of Validation Study Subjects

The activity of the IDEFICS subjects was low, regardless of BMI z-score. There was found to be no correlation between BMI z-score and physical activity levels and time spent being sedentary. When including all centres the correlation was low (Figure 10) so it was suggested to remove Gothenburg as this centre used subjects from an obese or overweight clinic. Even after removing these subjects, there remained no correlation between BMI z-scores and physical activity. Further supporting studies mentioned previously in this thesis that the physical activity levels of children are very low regardless of BMI. In a contrasting study by Ojiambo *et al*., (2010) the impact of urbanisation was investigated on objectively measured physical activity levels, sedentary behaviour and indices of adiposity in Kenyan adolescents. This was very similar study to our current one, with physical activity being measured using accelerometers and BMI z-scores used to assess adiposity. In the Kenyan study, there were significant differences in daily time spent sedentary between rural vs. urban male subjects, with 678 ± 95 vs. 555 ± 67 min sedentary, respectively; $P<0.001$) Rural males also spent more time in MVPA than urban males, (68 ± 22 vs. 50 ± 17 min, respectively; $P<0.01$). Time spent in sedentary behaviour was significantly different between rural females and urban females: (539 ± 91 vs. 694 ± 81 min, respectively; $P<0.001$)., and rural females partaking in more MVPA compared to urban females (62 ± 20 vs. 37 ± 20 min, respectively; $P<0.001$). It was also found that there was there was a direct association between physical activity, sedentary behaviour and adiposity in the Kenyan adolescents, which can be seen in Figure 12. Kenyan adolescents with low BMI took part in more MVPA than those who had a high BMI, and the higher the BMI score of the subject, the more time spent in sedentary activities (Figure 12). These findings were not seen in the current IDEFICS study. It is also not surprising that a higher percentage of the Kenyan cohort met current physical activity guidelines. Of the Kenyan subjects, 55% of the rural adolescents met current guidelines, which was significantly higher than the 17% of urban adolescents. Even though this is much lower than the rural subjects, the urban adolescents had a higher compliance than the IDEFICS cohort, where none of the subjects met current guidelines. The guidelines are set as minimum amount of time spent in MVPA, both to maintain a healthy weight and to stay healthy, but they are set for

children who are of a normal BMI. Therefore if the child is overweight or obese, like with some of the IDEFICS subjects, the time spent in MVPA should increase so that they can achieve similar health benefits through physical activity. As none of the IDEFICS subject met the current guidelines, this might prove problematic to achieve for any of the subjects.

Figure 11: Taken from Ojiambo et al., (2010). Figures show Pearson correlation coefficient of average CPM, % sedentary and % MVPA vs. BMI z-scores in Kenyan adolescents



4.4 Limitations

The present study is not without limitations; which include lack of a criterion measure of physical activity assessment such as direct observation. This limits our ability to recommend the most appropriate epoch and cut-points to relate to physiological outcomes such as energy expenditure. The manual analysis partly relies on parents recording ON/OFF times of the accelerometer so if they forget or input the wrong time, it effects the outcome of that particular time. However, the results still show that the results from manual and automated are in agreement, with few differences in minutes between. Secondly, while determination of reliability of accelerometer outcome variables is useful to accurately and reliably assess physical activity and sedentary time across a variety of populations and measurement protocols, applying any of these target number of days to all studies of physical activity and sedentary time in children will have inherent limitations (Trost *et al.*,2004; Olds *et al.*,2007). The sample-specific nature of the ICC has been demonstrated in a number of PA studies (Trost *et al.*,2004; Olds *et al.*,2007). This is because the magnitude of the intra- and inter-individual variance in physical activity is specific to the population 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 (Trost *et al.*,2004).

Conclusions

The main conclusion drawn from this study is that methodological issues in the analysis of accelerometer data have significant effects on the outcome variables. Epoch and cut-points have a significant effect on sedentary and MVPA classification. The effect varies depending on the cut-points and epoch selected. It is therefore emphasized that for ease of comparison between studies, a consensus should be achieved on the choice of epoch and cut-points used to assess physical activity and sedentary time in children. Furthermore, at least 6-hr of 7-9 days of monitoring and including at least 1 week-day would appear to be necessary to assess reliably physical activity and sedentary time in young children

Appendices

Appendix 1: Ethical approval form

Appendix 2: Validation study information pack for parents and children

Appendix 3: Example of R Summary output file

Appendix 1: Ethical Approval Form

UNIVERSITY OF GLASGOW

FACULTY OF BIOMEDICAL AND LIFE SCIENCES

ETHICS COMMITTEE FOR NON CLINICAL RESEARCH INVOLVING HUMAN SUBJECTS, MATERIAL OR DATA

APPLICATION FORM FOR ETHICAL APPROVAL

NOTES:

A submission to this Committee does not automatically result in approval. Investigators must wait for written approval before commencing data collection. Disciplinary measures will be taken if work commences without ethical approval being in place. The matter will be referred to the Dean for appropriate action.

THIS APPLICATION FORM SHOULD BE TYPED, NOT HAND WRITTEN.

ALL QUESTIONS MUST BE ANSWERED. "NOT APPLICABLE" IS A SATISFACTORY ANSWER WHERE APPROPRIATE.

Project Title: **Validation of field measurements of energy expenditure, physical activity and body composition assessment methods in young children.**

_____ *Is this project from a commercial source?* ***No***

If yes, give details and ensure that this is stated on the Informed Consent form.

Date of submission: **February 2007**

Name of all person(s) submitting research proposal: **Dr Yannis Pitsiladis**

Position(s) held: **Reader in Exercise Physiology**

Division: **CAMS**

Address for correspondence relating to this submission: **Lab 245, West Medical Building. Phone: 0141 330 3858, email: Y.Pitsiladis@bio.gla.ac.**

1. Describe the purposes of the research proposed.

The environment of infants and children has drastically changed in Europe during the last decades resulting in an increased development of overweight, obesity, metabolic syndrome, type II diabetes and musculoskeletal disorders (Reilly *et al*. 2002). To stop the epidemic of diet- and lifestyle-induced morbidity in European children, an integrated project (IP) entitled, " The identification and prevention of dietary- and lifestyle-induced health effects in children and infants" (IDEFICS) will be undertaken by an international consortium lead by the University of Bremen, Germany (www.idefics.eu). This project will (1) enhance the knowledge of the health effects of a changing diet and an altered social environment and lifestyle of infants and children and (2) develop, implement and validate specific intervention approaches, focusing on the age group of 2 to 10 years.

At present there is a paucity of data examining the accuracy and repeatability of field measures of energy expenditure (TEE), physical activity, and body composition in very young children. Therefore, a validation study must be completed, which will allow an appraisal of the techniques proposed for the main survey periods against "gold standards" or reference methods. For this validation study, the reference methods have been defined as the Doubly Labelled Water technique (DLW) for measuring TEE and a three- or four-component model for assessing body composition. Based on the results of the validation study, the most accurate and reliable technique for each measure will be selected and implemented in the main survey. Therefore, the aims of this validation study are:

1) To compare predictions of TEE and AEE (energy expended in physical activity) obtained from waist-mounted uni-axial accelerometers (GT1M ActiGraph^{TM}, Fort Walton Beach, Florida, USA and Actiband, Cambridge Neurotechnology, Cambridge, UK), a tri-axial accelerometer (3dNX^{TM} BioTel, Bristol, UK), a wrist-mounted uni-axial accelerometer (Actiband, Cambridge Neurotechnology, Cambridge, UK), an ankle-mounted uni-axial accelerometer (Actiband, Cambridge Neurotechnology, Cambridge, UK), and a uni-axial accelerometer combined with a heart rate sensor (Actiheart, Cambridge Neurotechnology, Cambridge, UK) with a direct measurement of TEE and AEE derived by DLW. The accelerometer providing the most accurate prediction of TEE and AEE in free-living children will be selected for use in the main survey.
2) To define cut-off points for accelerometry output that differentiate sedentary, light, moderate and vigorous physical activities in both the uni-axial and tri-axial accelerometers, enabling the length of time each child spends at each intensity to be quantified in the main survey.
3) To compare and contrast outputs from field-based methods of body composition relative to the three- and four-component models to determine the most appropriate measurements for use in the main survey.

2. Please give a summary of the design and methodology of the project. Please also include in this section details of the proposed sample size, giving indications of the calculations used to determine the required sample size, including any assumptions you may have made. (If in doubt, please obtain statistical advice).

Methods/Design of investigation

We propose to study 100 children**,** with an equal number of boys and girls spanning the age range of 4-8 years (this sample size is in line with the statistical procedures to be used). Researchers at the University of Glasgow, UK (UGLW), the University of Zaragoza, Spain (UZAZ) and the University of Ghent, Belgium (UGENT) will each test one third of the cohort of children (Table 1). Ethical approval will be sought from the local ethics committee of each research institution. Subjects will be in good health at the time of testing. Any child suffering from any physical or mental handicap will not participate in the study. Any child who receives an injury limiting physical movement will be excluded from study participation (e.g. broken arm, leg). The parents or guardians of each child will be interviewed in the presence of the child to assess suitability to participate in the study. The parents or guardians will also be required to read and sign the enclosed information sheet.

Table 1. Validation centres to offer numbers of children in each age and gender cell

Partner	**Boys, 4-8 years**	**Girls, 4-8 years**
UGLW	16	16
UGENT	17	17
UZAZ	17	17
Total	50	50

Protocols

Each child will participate in a 7-day monitoring period, during which the following measurements will be recorded. The parents/guardians or teachers of each child will be actively encouraged to attend as many measurement sessions as possible.

DLW. Doubly labelled water (DLW) will be used to determine TEE over a proposed 7-day assessment period. DLW will be centrally purchased by the University of Glasgow. Professor Klaas Westerterp, Chair of Human Energetics at Maastricht University , The Netherlands, will be appointed from the University of Glasgow to undertake the urinary analyses and assist in the interpretation of the data.
The principle behind this method is well described by Ainslie et al. (2003). Each child will be given a single oral dose of DLW in the morning (i.e. 10 atom percent excess 18-Oxygen and 5 atom percent excess 2-Hydrogen). A baseline urine sample should be collected (sample 1) in the evening of Day 0 and the time noted. Subsequently, the DLW should be ingested by the child (after consumption, the bottle should be rinsed with tap water and ingested again) and the time of ingestion noted. This should be the last consumption of the day. To evaluate the isotopic decay in body water, urine samples will be collected on days 1 (2^{nd} void and subsequent void), 4 (1^{st} and subsequent void) and 8 (1^{st} and subsequent void) in a dry plastic container. Parents should be requested not to rinse out containers prior to collection. The plastic containers should then be kept in the freezer until the final day of the measurement period. 2ml from each urine sample should be transferred into 2 individual glass vials (labelled with subject and sample number) and kept frozen at -20^{o}C until the end of the data collection period. 1 glass vial from each urine sample should remain in the research centre and the other sent to Maastricht for analysis. Urine samples will be sent as one batch directly from all centres to the central laboratory at the end of data collection. Young children that do not yet have full control over their urination may need to use modified nappies. Urine samples will be analysed by isotope ratio mass spectrometry with an analytic precision of 0.2 ppm for ^{2}H and 0.4 ppm for ^{18}O. The value of 0.85 will be used to estimate of the respiratory quotient, based on the

where: BW is body weight

Methodology (continued)

consumption of a standard Western Diet (Ainslie *et al.*,2003). TEE will be calculated according to Schoeller *et al.*,(1986). Because the Hydrogen and Oxygen isotopes used in the DLW test are non- radioactive, and also non-toxic in the doses used, the DLW measurement of TEE has been used extensively in human volunteers, and even in infants (Jones et al. 1987) and pregnant women (Heini et al. 1991). Measurement of total body water (TBW) will be done using the water labelled with 5 atom percent excess 2-Hydrogen. The same baseline and daily urine collection procedure will apply, so the samples collected will be used for both body composition and assessment of TEE.

Three-component model for measurement of body composition (reference model A). A three-component model incorporating TBW, actual body volume (ABV) and fat mass (FM) is considered as the reference method for body composition assessment in children (Wells et al. 1999). The BOD POD™ (Life Measurement Inc., Concord, CA, USA) will be used to measure ABV after adjustment for predicted lung volume (LV) and surface area artefact (SAA) (Dewit *et al.*,2000). The BOD POD™ consists of two chambers separated by a moulded fibreglass seat. The door is located at the front chamber and includes a large acrylic window, creating a comfortable and open environment. By oscillating the volume of air in the two compartments and thus altering pressure, body volume can be derived using Boyle's Law ($P_1/P_2=V_2/V_1$). Measurements of body volume will be made in triplicate and with subjects in swimwear, wearing a swimming cap, and with all jewellery removed. Measurements take 20 seconds to complete, during which time the subject will be required to remain still. Each subject will be seated in the chamber for no longer than 4 minutes in total and will only be measured on 1 occasion. The BOD POD™ system has been used to test a wide variety of individuals (5-90 years old) and is preferable to under-water weighing which is time-consuming, often considered unpleasant and/or difficult by subjects and requires considerable technician training.

FM will be derived from ABV and TBW as follows (Wells et al. 1999):

$$FM(kg) = [(2.22 \times ABV)-(0.764 \times TBW)]-(1.465 \times BW)$$

Where: BW is body weight

Four-component model for measurement of body composition (reference model B).- DEXA. Due to the paucity of data in very young children, body composition will also be measured using DEXA. Each subject will be required to lie supine on an X-ray table for 10-15 min while two X-ray beams with differing energy levels measure body fat, muscle, and bone mineral. The principle of the method is that soft tissue and bone attenuate X-rays to different degrees. The results may be viewed as whole body estimates of body fat, muscle, and bone mineral as well as regional body estimates. DEXA does involve a small amount of radiation although this is only 1/30 of the radiation dose received during a standard X-ray and will be administered by clinical personnel qualified to make use of radiation for medical imaging. Each subject will only be measured on 1 occasion.

FM will be derived from ABV and TBW as follows (Fuller et al. 1992):

$$FM(kg) = [(2.747 \times ABV)-(0.710 \times TBW)] + [(1.460 \times A) – (2.050 \times BW)]$$

Where: A is bone mineral content determined by DEXA (in kg). Total-body mineral mass is calculated as BMC x 1.2741(Brozek et al. 1963).

Resting or basal energy expenditure (REE). When possible REE will be measured by indirect calorimetry using a metabolic hood (Delta-Trac). Each subject will be required to lie comfortably on a flat surface for 15 minutes with a clear plastic hood is placed over the head and upper body. Each subject will only be measured on 1 occasion. REE is required in order to accurately determine TEE (i.e. TEE = REE + AEE + DEE). Where AEE is energy expended in physical activity and DEE is the thermic effect of food or diet-induced energy expenditure. The DEE can last some hours after a meal but is relatively small (5-10% of daily energy intake). DEE will be ignored or estimated (Hayes *et al.*,2005), rather than measured in the present validation study. The Schofield equations adopted by FAO/WHO/OMS 2001 will also be utilized to estimate basal metabolic rate (BMR) in children who are non-compliant with the metabolic hood procedure.

Methodology (continued)

The equations are based on body weight and are specific to 3-10 year old girls and boys (Schofield, 1985).

Energy expended in physical activities (AEE). AEE refers to EE from all activities (i.e. AEE = TEE – (REE + DEE)). AEE will be measured in a separate cohort of 20 children per age group (ideally 10 boys and 10 girls) using indirect calorimetry and accelerometers during periods of rest and activity (see physical activity section below).

Body composition. FM will be estimated using skinfold callipers (Harpenden, UK) and an handheld ultrasound scanner (Biometrix, Germany) according to the two skin site method (i.e. triceps and subscapular; Wells *et al.*,1999, Reilly *et al.*,1995 using the equations of Slaughter *et al.*,1988), four skin site method (biceps, triceps, subscapular, suprailiac; Durnin and Wormsley, 1974), the six site method (biceps, triceps, subscapular, suprailiac, thigh and calf) on 1 occasion. The skin is pinched at the appropriate site and the layer of subcutaneous fat measured with the callipers. After application of a small amount of electrode gel on the surface of the skin, the ultrasound scanner is then placed over the same site for approximately 5 seconds. Male investigators will only measure FM in male children and likewise for female investigators. FM will also be assessed using biolectrical impedance (Tanita BC 420 and/or Bodystat 1500MD, Bodystat Ltd., Isle of Man, UK). This non-invasive method involves placing two current-inducing electrodes and two detector electrodes on the dorsal surfaces of the right hand and foot and a small (and imperceptible) electrical current (500 Micro-Amps) introduced between these (Ross et al, 1989). The following circumferences will also be determined: waist, hip, neck, mid-upper arm (and waist/hip ratio determined) using a standard measuring tape.

Physical Activity. *Physical activity or AEE will be measured by comparing and contrasting the output from waist-mounted uni-axial accelerometers (GT1M ActiGraph™, Fort Walton Beach, Florida, USA and Actiband, Cambridge Neurotechnology, Cambridge, UK), a tri-axial accelerometer (3dNX™ BioTel, Bristol, UK), a wrist-mounted uni-axial accelerometer (Actiband, Cambridge Neurotechnology, Cambridge, UK), an ankle-mounted accelerometer (Actiband, Cambridge Neurotechnology, Cambridge, UK) and a uni-axial accelerometer combined with a heart rate sensor (Actiheart, Cambridge Neurotechnology, Cambridge, UK) and quantifying their relationships with TEE derived by DLW. Each accelerometer will be worn during all waking hours (except when swimming or bathing) during a 7-day assessment period (i.e. the same 7 days as DLW assessment). A high compliance to wearing the units throughout the day is essential so routine prompts and checks by the local research team, the teachers and parents will be completed. The waist-mounted Actiband accelerometer is worn on an elasticised strap that will also contain a pouch hosting the Actigraph and 3dNX accelerometers. The wrist- and ankle-mounted Actiband accelerometers will be attached to a plastic strap and worn on the dominant arm and the ankle of the dominant leg. The Actiheart is attached to the chest with two standard ECG electrodes. One electrode is placed at the base of the child's sternum and the other horizontally to the child's left side, with the Actiheart spaced so that the wire between the two sections of the Actiheart is straight but not taut. Body movement (counts) will be recorded in 5-second epochs due to the short duration burst activities characteristic of child behaviour. 3dNX™ data output is in the form of individual axis counts and total counts (x, y and z axes combined) per epoch. ActiGraph, Actiband and Actiheart output is in the form of counts for the single axis. All data will be averaged over the 7 day period and expressed as activity counts per day (ACD – total 7 day count divided by 7). Only subjects who have worn the units for at least 80% of their waking day will be included in the data analysis and the period of time worn used as a co-factor in the analysis. Parents will be asked to keep an activity/sleep diary so that total waking hours, and compliance can be calculated. Additional data on the times spent in various modes of transport (pushchair/buggy, bicycle, car, train) and the times and reasons that the accelerometer was not worn will also be included in the activity diary. A physical activity diary for the full 7-day period will to be completed at home by the parent and a Burdette energy expenditure questionnaire will be*

Methodology (continued)

To determine accelerometer cut-off points and measure activity energy expenditure for different physical activities, a separate cohort of 60 children (ideally 30 boys & 30 girls) will be recruited. Each centre will measure 20 children. Following the collection of several basic descriptor variables (age, height, weight, sex), a number of accelerometer units and a portable metabolic recorder (K4) will be attached to the children and a sequence of pre-identified common activities will be undertaken in 5 minute bouts. The K4 metabolic system consists of a facemask and recording device strapped to the back and will provide a direct measure of AEE during the various activities, to which the accelerometer predictions of AEE will be compared. The range of activities will encompass sedentary, light, moderate and vigorous events. For example: lying, sitting, slow walking, walking, jogging and 1 pre-identified activity (i.e. soccer, hopscotch, basketball). The list of staged activities will be further worked out by UGLW and provided to each of the validation study centres.

***Other measures**. Height (m) and body mass (kg) will be measured on two separate days prior to the start of the measurement period. Stature will be measured using a portable Seca Leicester Stadiometer (Seca Ltd, Birmingham, UK). Body mass will be measured on Seca Alpha 770 digital scales (Seca Ltd, Birmingham, UK). The equipment will be calibrated and the procedures conducted according to the manufacturers. Body mass will also be measured each morning of the 7 day assessment period by the parent using digital scales provided.*

3. Describe the research procedures as they affect the research subject and any other parties involved.

The research procedures require the subjects to participate in several anthropometrical and physiological measurements over a 7-day period at times convenient to them. The period of time taken for each measurement will vary, but should not exceed 30 minutes, with the exception of the determination of the accelerometer cut-offs, which will last approximately 60 minutes. The majority of measurements will require the child to be sedentary, but during the determination of accelerometer cut-offs, the children will experience feelings associated with performing exercise e.g. increased heart rate, increased rate of ventilation. During this exercise period, expired air will be collected using a K4 portable metabolic analyser using a face mask. This is invasive in the sense that the mask is worn over the mouth and nose so any expired air can be collected and not vented to the atmosphere. The facemasks we are proposing to use are specifically designed for paediatric use.

Actiheart accelerometers will be used to record each subject's heart rate and physical activity during the 7-day period. These are non-invasive and have been used extensively in children. The Actiheart is attached with two standard ECG electrodes that are replaced every 2-3 days. There is a possibility that participants may have an allergic reaction to the adhesive on the electrodes, so parents will be supplied with 3 manufacturer's varieties utilising different adhesives to minimise this risk. The other accelerometers (strapped round the waist, wrist and ankle on a belt) have been used in children as young as 3 years and have been well tolerated, with no side effects.

Each child will be asked to consume a small volume (less than 50ml) of doubly labelled water for determination of TEE and TBW. The water is clear, tasteless and cannot be distinguished form ordinary tap water. Because the heavy hydrogen and oxygen isotopes

4. What in your opinion are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

There is an issue of informed consent with the proposed participants in the study. They are of primary school age and may not fully comprehend the reasons, techniques or implications of being involved in the study. In accordance with the Central Office for Research Ethics Committees (COREC) guidelines, parents/guardians and children will be given an information pack. This pack contains separate information sheets for parents and children, written in as simple language as possible to make it clear for the children and parents. Parents and children will have at least 24 hours to discuss and consider participation or not. An opportunity for parents and children to ask questions will be given at the time of distributing information packs, visiting the lab or at any point throughout their involvement in the study (the principal investigator's contact details are given in the information packs to allow parents to ask questions). There are no consequences for the participants if they decide at any point, with or without reason that they do not want to take part in the study.

Any information about the participant, e.g. name, date of birth, height, weight, will be held confidentially. Information collected will be made available to the relevant participant's parents if requested. The risk to the participants is minimal.

5. Outline the reasons which lead you to be satisfied that the possible benefits to be gained from the project justify any risks or discomforts involved.

The alterations of behaviour, unhealthy dietary habits, and low physical activity levels in children throughout the European Union has led to significant increases in obesity, metabolic syndrome, type II diabetes and musculoskeletal disorders (Reilly *et al*. 2002). To stop the epidemic of diet- and lifestyle-induced morbidity in European children there needs to be enhanced knowledge of the health effects of a changing diet and an altered social environment and lifestyle of infants and children and implementation of a specific intervention approach, focusing on the age group of 2 to 10 years. Before such an intervention programme can be implemented, we must first identify and validate tools that can be used in the field to assess body composition, energy expenditure and physical activity. These measurements will provide aetiological data for each European country involved in the study allowing the direction of the intervention program to focus specifically on the probable causes of obesity within said country. Furthermore, the physiological and anthropometric assessments will provide a medium by which to assess the success or failure of any intervention programme that is carried out.

The minimal risk and discomfort associated with the above procedures are considered to be worthwhile to gain the information required.

6. Who are the investigators (including assistants) who will conduct the research and what are their qualifications and experience?

Dr Yannis Pitsiladis PhD MMedSci BA, Mr Chris Easton BSc, Dr Robert Scott BSc PhD, Miss Vasiliki Lagou BSc, Mr John Wilson (Senior technician), Mrs Heather Collin (Senior Technician). The principal investigators have wide ranging experience of exercise testing, including with children, over periods of up to 10 years without incident. All investigators will have passed Disclosure Scotland checks before working with children.

7. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

In the event of an emergency, guidelines recently approved by the ethics committee will be followed.

In the event of an untoward incident that is not an emergency, the supervising Principal Investigator will administer appropriate first aid, if necessary. The subject will not be permitted to leave the laboratory until he/she has fully recovered. The parents/guardians of the subject will be encouraged to contact his/her local GP. The parents/guardians will be told that one of the Principal Investigators will conduct a follow-up by telephone at the end of the same day. The parents/guardians will also be provided with 24-hour contact numbers for both Principal Investigators.

8. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital) describe the arrangements you intend to make to gain access to this information including, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

Participants will only be identified after an initial invitation to attend a presentation about the study (e.g. at after school club or in a school assembly). All those attending will receive an information pack. If they would like to participate, they return the consent forms and contact details sheet in the pre-paid envelope provided with the pack.

9. Specify whether subjects will include students or others in a dependent relationship.

Participants will be under the age of 18 and thus in a dependent relationship with a teacher/parent/guardian. Recruitment and informed consent procedures are in place to ensure the participants are aware they can withdraw from the study at any time, without consequence.

10. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects.

This research will involve children. The increasing obesity rates in children throughout the European Union are of huge concern and thus an intervention programme must specifically target individuals below the age of 16.

11. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering payment or other incentive.

No payment or incentive to take part will be offered.

12. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Parents and children will be recruited locally e.g. from schools or from parents within the University. After an initial invitation to participate, a presentation about the study will be made to children and their parents. All of the measurements that will be taken during the course of the study will be demonstrated during the presentation. Those parents and children who are interested will receive an information pack following the presentation. Parents and children will be encouraged to ask questions at any point in the recruitment and consent procedure. The information pack will contain: (1) welcome letter, (2) parental information sheet, (3) child information sheet, (5) parental consent forms (x2), (6) child consent forms (x2). There will be separate information packs for the main validation study and the small sub-study to determine accelerometer cut-offs. Both information packs are enclosed with this application. On completion of the presentation, parents and children will be invited to discuss their possible involvement in the study before deciding whether to take part. Participants can confirm consent at the presentation or by returning the consent form to the investigators by mail.

13. Comment on any cultural, social or gender-based characteristics of the subject which have affected the design of the project or which may affect its conduct.

The participants will be recruited from primary schools that cover all deprivation categories so no socio-economic or gender bias will exist in this study.

14. Please state who will have access to the data and what measures which will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g. will the data be anonymised?

All research group members (see page 1) will be involved in collecting these data and thus have access. The research team, from moment of recruitment and consent, will maintain confidentiality of the participant. Each participant will be assigned a research code by the investigators. The record matching the participant details with the code will be kept in electronic form in a locked filing cabinet. From the time the research code is applied to the analysis of the data, the participants will be referred to by this code. The participants will not be identified or will be referred to anonymously when presenting these data.

15. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

To our knowledge none of the intended group of research subjects will be involved in an other research studies.

16. Date on which the project will begin **May 2007** and end **November 2007**

17. Please state location(s) where the project will be carried out.

The majority of the proposed measurements will be carried out within the research participant's school or home.

The DEXA measurement will be carried out in Yorkhill Hospital, Glasgow and the BodPod measurement in the Royal Infirmary, Glasgow.

The resting metabolic rate measurement will be completed in the metabolic suite of the IDEAL laboratories, West Medical Building, University of Glasgow.

18. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken

All experiments will be conducted according to the code of practice for conducting

experiments in non-patient human volunteers previously accepted by the University Ethics

Committee.

Signed _____ Date _____

(Proposer of research)

Where the proposal is from a student, the Supervisor is asked to certify the accuracy of the above account.

Signed _____ Date _____

Supervisor of student)

Appendix 2: Validation study information pack for parents and children.

UNIVERSITY of GLASGOW

Information Pack for Parents and Children

idefics study
Learning healthy living

Dear parents,

As you may or may not know, the number of children who are overweight or obese has increased significantly over the last few decades, particularly in the west of Scotland. Of course, obesity during childhood can lead to many clinical complications during adulthood such as diabetes and heart disease. Whether the increasing obesity rates are due to an increase in unhealthy eating such as fast food and ready meals or the fact that kids don't exercise as much as they used to, is unknown. However, the European Union feels that the obesity epidemic is so worrying, they have funded a major study to identify what is causing children to become overweight so that an intervention strategy can target the specific problem area. The project is entitled: The identification and prevention of dietary and lifestyle-induced health effects in children and infants (IDEFICS) and will assess 17,000 children in 10 different countries all over Europe. However, before this project can begin, we must decide what methods we will use to assess the children's health, fitness and body composition throughout the European Union. Obviously, 17,000 is a lot of children, so the methods used in the IDEFICS study must be quick, easy to use and provide an accurate measure of the child's health.

How you can help?

Therefore, we are asking that your child participates in a small research study, where the methods we wish to use in the large study across Europe will be compared to the so called 'gold standard' methods, such as those found in a hospital. 33 children from Glasgow will participate in the study, with the same number participating in Zaragoza, Spain and Ghent, Belgium. All of the methods we intend to use in the study are used regularly in children and will not cause pain or discomfort of any kind. Your participation is completely voluntary and even if you decide to take part you can withdraw your child at any point without having to give an explanation. Your child's data will be made completely anonymous and they will never be referred to by name in any publication (each child is assigned an identification number). Only you, or your child will have access to the data, and we will happily go over your child's individual results with you, should you wish. Naturally, such a large-scale project can only work with a little bit of help – from the schools and

nurseries and especially from the parents. What we need is your interest, your readiness to help and your engagement. Only if we begin to learn to understand health, can we improve the future for our children.

If you are interested in taking part in the study, please read the information sheet for parents on the next page and read aloud the information sheet for kids to your child. Please then sign the consent forms (both parent and child forms) in duplicate and return to one to us directly or via post to the address at the bottom of the sheet, keeping one copy for yourself at home. We will then contact you with further details.

Yannis Pitsiladis Chris Easton

University of Glasgow

Institute of Biomedical and Life Sciences
University of Glasgow

PARENT'S INFORMATION SHEET

Study title: Validation of field measurements of energy expenditure, physical activity and body composition assessment methods in young children.

Your child is being invited to take part in a research study. Before you decide whether your child will participate, it is important for you both to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study? The environment of infants and children has drastically changed in Europe during the last decades as reflected in alterations of behaviour, unhealthy dietary habits, and low physical activity. Dietary as well as lifestyle factors appear to play a part in the development of overweight, obesity, metabolic syndrome, type II diabetes and musculoskeletal disorders. To stop the epidemic of diet- and lifestyle-induced morbidity in European children, an integrated project (IP) entitled, "The identification and prevention of dietary- and lifestyle-induced health effects in children and infants" (IDEFICS) will be undertaken by an international consortium lead by the University of Bremen, Germany (www.idefics.eu). This project will (1) enhance the knowledge of the health effects of a changing diet and an altered social environment and lifestyle of infants and children

and (2) develop, implement and validate specific intervention approaches, focusing on the age group of 2 to 10 years. The study is designed to run for five years and is funded by the European Commission (DG Research). 24 renowned research institutes and small and medium sized enterprises located in 10 different EU-countries are participating in the IDEFICS-Study, which commenced on September 2006. Surveys will help to assess the prevalence of overweight, obesity, metabolic syndrome, diabetes (type II) and related risk factors. Promotion and prevention modules will be implemented and evaluated in nurseries and schools in eight European countries in order to develop efficient evidence-based approaches. The project will provide a knowledge-based set of guidelines on dietary, behavioural and lifestyle activities for health promotion and disease prevention in children for scientists, health professionals, policy makers, stakeholders, channels, and consumers at a pan European level and for individual countries. At present there is only small amounts of data examining the accuracy of field measures of energy expenditure, physical activity, and body composition in very young children. Therefore, a validation study must be completed, which will allow an appraisal of the techniques proposed for the main IDEFICS study against "gold standards" or reference methods.

Why has your child been chosen? Your child has been selected as a possible participant in this investigation because they are aged 4-8, are in good health and do not suffer from any mental or physical handicap, or injury limiting physical movement. One hundred volunteers are being sought overall and 33 will be recruited in Glasgow.

Does your child have to take part? It is up to you and your child to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to your child if they take part? Each child will participate in an 8-day monitoring period, during which several measurements will be made on your child. You are actively encouraged to attend as many of these measurement sessions as possible. A researcher from the University of Glasgow will stay in contact with you throughout the measurement period and will be present during all the measurements. A female researcher will always perform measurements on a female child and vice versa for the male children. All of our researchers hold current disclosure Scotland certificates and have extensive experience in working with children.

Doubly labelled water: On the night before the first day of the monitoring period, we will ask your child to drink 50ml of doubly labelled water. Although this sounds a bit strange, it is just normal tap water with a special formula. However, when your child goes to the toilet, we can measure how much of the urine is normal water and how much of the urine is doubly labelled water. These measurements will then tell us how much water your child has in their body and also how much energy they are using up over 7 days. The doubly labelled water tastes exactly like tap water and will not cause harm to your child in any way. Doubly labelled water is used very regularly for measurements in babies, children and even pregnant women and has never caused any bad reactions or side effects, and is our 'gold standard' measurement of energy expenditure. Below you can see a woman drinking some of the water. We will be on hand (we can travel to your home to make things easier) with you to make sure your child drinks the full 50ml.

Woman drinking doubly labelled water.

To allow us to work out how much energy your child is using, we will need to ask you to collect some urine samples from your child. We will need you to collect a sample once on the night before the monitoring period, then once on days 1, 4 and 8 of the monitoring period. We just require a very small amount of urine on each day. We will supply you with several urine collection containers (like the one pictured below), which are sterile and will be frozen after the urine has been collected to allow us to analyse it at a later date.

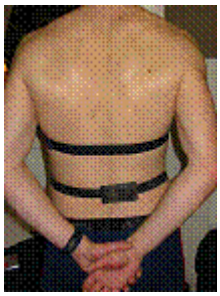
Urine collection container

Height and weight: On the morning of the first day of the monitoring period, we will measure your child's height and weight using normal bathroom scales and a simple measuring device like the ones shown below.

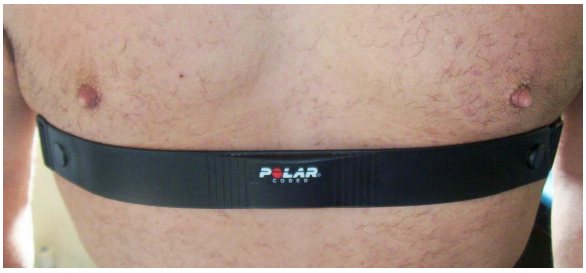
Scales and stadiometer for measurement of child's height and weight.

Accelerometers: These are very simple devices that we will attach to your child for the 7-day monitoring period. These are mini recording devices that measure movement, and are similar to the pedometers (step counters) you may have seen advertised on the television and in magazines. There are 2 different types of accelerometer we will ask your child to wear, as we want to find out which provides the best estimate of energy expenditure compared to what we find from the doubly labelled water. These devices will be contained on one elasticated strap that is worn round the waist like a belt. We will also ask your child to wear an elasticated strap around the chest that will record heart rate. The devices are extremely light and are designed so that they will not interfere with your child's normal movement. We would your child to wear the devices all the time for the 7-day period if possible including exercise (except for in bed and while bathing/swimming) and will ask you to record when the devices are put on and taken off each day. For your interest, photos of each device can be seen below.

Waist-mounted accelerometer 1: Actigraph



Waist-mounted accelerometer 2: 3dNX



Heart rate strap

Body fat measurement: At some point throughout the 7-day monitoring period we will measure the body fat at 6 different sites on your child's back and arms using two devices: skinfold calipers and an ultrasound device. These measurements are very quick and will take no longer than 15 minutes in total to complete and can be performed in a location convenient to you. A researcher will lightly pinch the loose skin at each of the different sites and measure the thickness of this with the skinfold calipers (see picture below). The utmost care will be taken not to cause any pain or discomfort to your child during these measurements. At the same 6 sites, a portable ultrasound scanner will be used to measure the layer of fat between the skin and the muscle (see picture below).

This device works like the ultrasound machine used to scan babies in the womb, and will not cause any pain or discomfort. We will also measure the size of your child's waist, neck, thigh and hip using a standard measuring tape (see below).

Skinfold calipers

Ultrasound scanner

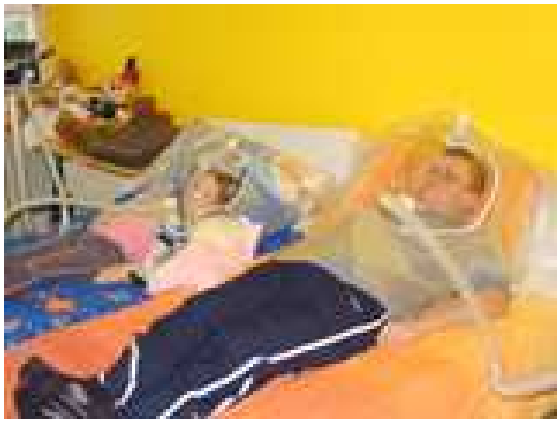
Waist size measurement

DEXA: At some point throughout the 7 day monitoring period, we will arrange one visit for you and your child to Yorkhill Children's Hospital, Glasgow. The purpose of this visit is to perform a whole body scan on your child using a method called dual energy X-ray absorptiometry (DEXA). The scan is very simple, and merely requires your child to lie flat on a hospital bed for

about 10 minutes (See below), and will provide us with details like percentage body fat. The scan is like having an X-ray performed at hospital for a suspected broken bone, but gives out only 1/30 of the radiation dose received during a standard X-ray and will be administered by clinical personnel qualified to make use of radiation for medical imaging. The researchers will be present with you during the scan.

DEXA scanner

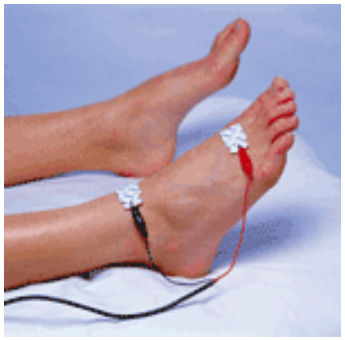
Resting metabolic rate: On one morning throughout the 7 day period, we will arrange to perform a measurement of resting metabolic rate. This is another simple measurement procedure, which requires your child to lie flat for around 30 minutes while we measure the amount of air that they breathe out. This measurement will let us know how much energy your child uses when they are resting and sleeping. We will place a clear plastic hood over the child's head (a bit like a spaceman's helmet, see picture below) while they watch the television/video. We can arrange to perform this measurement in your own home, or in the metabolic suite at Glasgow University.



Father and daughter during resting metabolic measurement

Bioimpedance: At some point throughout the 7-day period, we will measure your child's body water levels using 2 bioimpedance devices. The first simply requires the child to stand on a set of scales for 10 seconds, with their shoes and socks off while the machine takes the measurement (see picture below). The second requires the child to lie flat for about 5 minutes while we attach sticky pads to one hand and one foot (see pictures below). We then attach the pads to the machine and take the measurement. The bioimpedance devices pass a tiny current between the feet in the 1st device and between the hand and foot in the 2nd device. This current is so small the child will not feel anything and will not even be aware when the machine takes a measurement.

Bioimpedance device 1: TANITA



Bioimpedance device 2: Bodystat

Bodpod: On the final day of the 7-day monitoring period, we will arrange for you and your child to visit the Royal Infirmary in Glasgow for a measurement of body volume using a BOD POD™. The BOD POD™ consists of two chambers separated by a moulded fibreglass seat (see diagram below). The door is located at the front chamber and includes a large clear window, creating a comfortable and open environment. By slightly changing the volume of air in the two compartments body volume can be measured using a simple equation. Although the child will not be able to notice any change in air volume, the machine makes a quiet whirring sound. Measurements of body volume will be made 3 times while the child is seated comfortably wearing swimwear, a swimming cap, and with all jewellery removed (see picture below). Measurements take only 20 seconds to complete, during which time the child will be required to sit still. You and your child can sit in the BOD POD together for a few practise runs before we take measurements to allow your child to become comfortable with the procedure

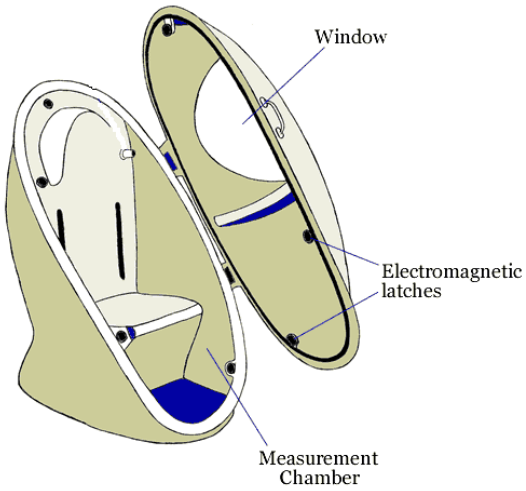


Diagram of BOD POD

Child in BOD POD prior to measurement

Questionnaires: *We will ask you to complete a very simple physical activity diary for the 7-day monitoring period on your child's behalf, which will let us know how often and what type of physical activity your child did during the week. At the end of the monitoring period we will also ask you and your child some simple questions about what type of physical activities your child does in a typical week. This should take no longer than 5 minutes.*

What are the possible disadvantages and risks of taking part? On very rare occasions some children have had an allergic reaction (mild rash) to the stick pads used to keep the Actiheart accelerometer attached to the chest. However, the symptoms

disappear very quickly after removing the pads. To lessen the chance of this happening, we will supply you with 3 different brands of sticky pads, so if a reaction does occur the pads can be replaced by others.

What are the possible benefits of taking part? The study will provide a comprehensive measurement of your child's physical activity levels and body composition allowing us to assess and advise you personally whether your child meets the government recommended guidelines. The majority of measurements will be 'made fun' by the researchers allowing your child to enjoy taking part in the research study. The results of the study will also allow us to design and implement a large-scale intervention study across the whole of Europe to try and reduce the number of overweight and obese children. The healthy future of our children and our children's children is dependent on us acting now.

What if something goes wrong? If you feel that you or your child are uncomfortable with any of the procedures during the study, **you can withdraw at any point, without having to give any reason.** In the highly unlikely event that your child is harmed by taking part in this research project, there are no special compensation arrangements. If your child is harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified are fully trained in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff are on hand. You may want to consult your GP if your child experiences any side effects from taking part in the study and should also inform the Principal Investigator.

Will my taking part in this study be kept confidential? All information about your child that is collected during the course of the research will be kept strictly confidential

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

Suggested Summary of monitoring period:

Day 0: Before bed-time a baseline urine sample collected (sample 1) in the evening of Day 0 and the time noted. Subsequently, the DLW should ingested by the child (after consumption, the bottle should be rinsed with tap water and ingested again) and the time of ingestion noted. This should be the last food/drink of the day. Researchers will arrange a time to meet the following morning and activate accelerometers to begin data collection at this time.

Day 1: Body mass and height will be recorded in the morning of Day 1 following the 1st urine void of the day. All accelerometers will be connected to the child by the researchers and the maintenance instructions given to you. The 2nd urine void of the day should be collected (sample 2) and the time noted and a further void collected later in the day, again recording time of collection (sample 3).

Day 2:

Day 3: Body fat measurements will be recorded using both calliper and ultrasound devices according to the ISAK protocol. Following this, limb girths (4 sites) will also be recorded using a Waist watcher measuring tape. The researchers will complete all anthropometric measurements. **[These measurements can be recorded at any time on Days 0-8]**

Day 4: The 1st urine void of the day should be collected (sample 4) and a further void collected later in the day (sample 5). The time both urine samples are collected should be recorded.

Day 5: Body composition will be assessed using DEXA at Yorkhill Children's Hospital. **[This measurement can be recorded any time on Days 4-8]**

Day 6:

Day 7: Following an overnight fast, resting metabolic rate will be measured using a metabolic hood for 15 minutes either in the child's home or in the lab. If it is not possible to measure RMR, then it will be estimated using the Schofield equations **[This measurement can be recorded any time on Days 4-8].**

Day 8: Following an overnight fast, the child will report to the Royal Infirmary. The 1st urine void of this day should be collected (sample 6) and all accelerometers should be removed. Following this, body mass will be measured, body water assessed using bioimpedance and body volume measured using Bod Pod. Researchers will then complete the physical activity questionnaire during an interview with you and your child.

If you wish to find out more about this investigation, you can contact:

Dr Yannis Pitsiladis

Institute of Biomedical and Life Sciences
West Medical Building
University of Glasgow
Glasgow, G12 8QQ
Phone: 0141 330 3858
Fax: 0141 330 6542
e-mail: Y.Pitsiladis@bio.gla.ac.uk

or

Dr Chris Easton
Institute of Biomedical and Life Sciences
West Medical Building
University of Glasgow
Glasgow, G12 8QQ
Phone: 0141 330 5055
Mobile: 07811595473
Email: C.Easton@bio.gla.ac.uk

Parent's Consent Form

I .....

Relationship to child .....

Child's name .....

give consent to allow my child to participate in the research procedures which are outlined above, the aim, procedures and possible consequences of which have been outlined to me

Signature ……

Date ……

University of Glasgow

Institute of Biomedical and Life Sciences
University of Glasgow

CHILD'S INFORMATION SHEET

Study title: Validation of field measurements of energy expenditure, physical activity and body composition assessment methods in young children.

Parents: Please read this information sheet aloud to your children.

We would like your help! Lots of children in different countries are getting fatter and fatter because they eat bad things and don't do enough exercise. We would like you to help us do a study to find out how we can help these other children. We have asked you, because you are aged between 4 and 8 years old and you live in Glasgow. If you don't want to take part in the study or if anything upsets you or annoys you, then please tell Mummy or Daddy and you will not have to take part any more.

Special Water: One night we will come to your house and ask you to drink a small cup of water like the lady in the picture below. This water just tastes like normal tap water, but is very special and helps us work out how active you are!

Woman drinking special water.

Pee samples: On four different days, when you got to the toilet, we would like you to pee into a cup like the one in the picture below. Don't worry about doing this on your own, Mummy or Daddy will be able to help you!

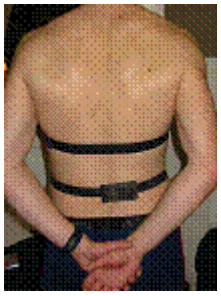
Pee cup

Height and weight: One day when your at school or nursery, we will ask you to step on some scales to see how heavy you are and also measure how tall you are, like the girl in the picture below.

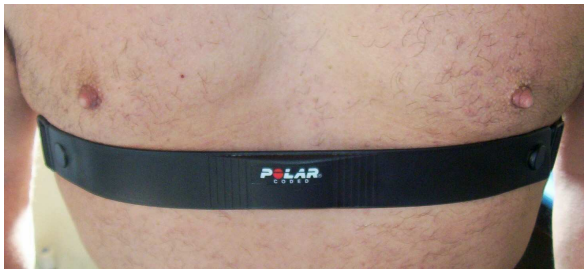
Weight and height measurement

Accelerometers: For one whole week you will get to wear some miniature computers called accelerometers. Every time you move, these computers will measure it for us. 3 of them will go round your waist like a belt, 1 of them you will wear like a watch on your arm, 1 of them you will wear like a watch on your ankle and one of them we will stick to your chest with sticky pads. Try and wear these computers as much as you can except when you go to bed or go under water. If any of the accelerometers are not comfortable tell your Mummy or Daddy and they will take them off. You can show all of your friends at school or nursery!

Waist-mounted accelerometer 1: Actigraph



Waist-mounted accelerometer 2: 3dNX



Heart rate strap

Body fat measurement: One day we will come to your school or nursery and measure how much body fat you have. We will ask you to wear a vest so that we can measure the fat on your tummy and your back. One of us will use a device called callipers to measure how thick the skin is which will tell us how much fat there is. Don't worry, this doesn't hurt and we will even let you have a practise shot on us first! We will also do the same measurements with a little torch that just touches your skin. Again this does not hurt at all! We will also use a measuring tape to measure your waist, your neck, your leg and your hips.

Calipers

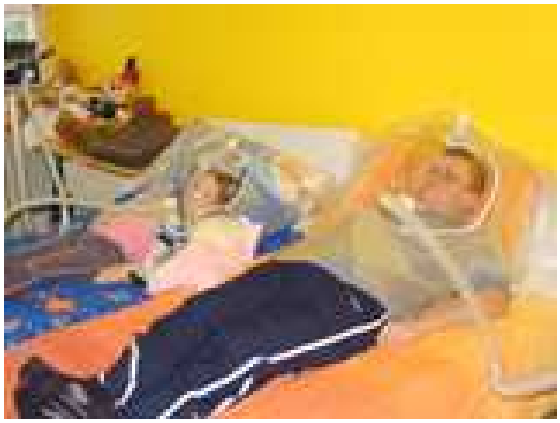
Torch

Measuring tape

DEXA: On one morning or evening we will bring you and your Mummy or Daddy to Yorkhill Children's hospital. Don't worry, the reason we are going there is because the hospital has a special machine that tells us how much water you have in your body. We will ask you to lie on the bed as still as you can for a few minutes, just like the girl in the picture below.

DEXA machine

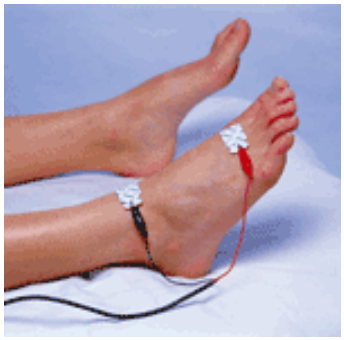
Resting metabolic rate: One morning we will come to your house and ask you to put a special space man's helmet on. This helmet is just like Buzz Lightyear's in Toy Story! This is a special helmet that lets us see how much air you breathe out. While we are measuring this, you can lie on your bed or sofa and watch some cartoons!



Buzz Lightyear helmets

Bioimpedance: On the same day, we will use some other machines that tell us how much water you have in your body. One machine you just step on like a set of scales and the other you just lie on your bed while we put some sticky pads on your hand and foot. These don't take any time and you won't even know we are taking the measurement!

Body water machine 1



Body water machine 2

Bodpod: On the very last day of the study we will ask you and your Mummy or Daddy to come to the Royal Infirmary Hospital. Again, this hospital has a very special machine that lets us see how big your body is! You will get changed into your swimwear and then sit in the special machine like the girl below. You need to sit still for about 10 seconds or so. We will have a competition to see who moves the lease amount, like playing musical statues! If you are unsure, your Mummy or Daddy can sit in the machine with you the first time.

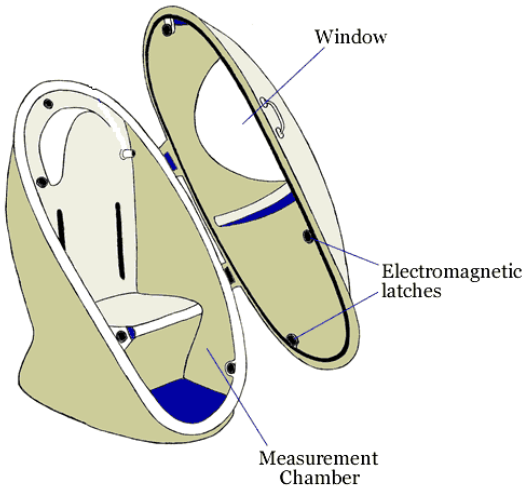


Diagram of BOD POD

Child in BOD POD prior to measurement

Child's Consent Form

I .....

give consent to participate in the research procedures which are outlined above, the aim, procedures and possible consequences of which have been explained to me

Signature ……

Date ……

Appendix 3: Example of R Summary output file

File	ID	Epoch	Period	Length	Wkdy	avg.cpm	tot.cnts	val.time	permax	Sirard.Sed	Sirard.Light	Sirard.Mod	Sirard.Vig	Sirard.MVPA
IV01.dat	1	60	07/12/2008	810	0	399.18	228730	573	6690	537	28	6	2	8
IV01.dat	1	60	08/12/2008	1440	1	521.09	352780	677	4167	618	55	4	0	4
IV01.dat	1	60	09/12/2008	1440	2	491.93	355175	722	3887	654	63	5	0	5
IV01.dat	1	60	10/12/2008	1440	3	624.18	435680	698	12801	612	73	8	5	13
IV01.dat	1	60	11/12/2008	1440	4	541.72	364579	673	8329	603	56	10	4	14
IV01.dat	1	60	12/12/2008	1440	5	736.57	592205	804	12366	695	81	14	14	28
IV01.dat	1	60	13/12/2008	1440	6	795.78	461555	580	13022	488	77	10	5	15
IV01.dat	1	60	14/12/2008	639	0	456.08	32382	71	3311	68	3	0	0	0
IV02.dat	2	60	07/12/2008	780	0	466.66	276732	593	3667	546	45	2	0	2
IV02.dat	2	60	08/12/2008	1440	1	631.13	493542	782	14541	693	61	18	10	28
IV02.dat	2	60	09/12/2008	1440	2	483.51	307032	635	4109	591	43	1	0	1
IV02.dat	2	60	10/12/2008	1440	3	464.70	301593	649	3686	601	47	1	0	1
IV02.dat	2	60	11/12/2008	1440	4	516.95	427514	827	5062	754	68	4	1	5
IV02.dat	2	60	12/12/2008	1440	5	461.27	419759	910	5159	835	70	4	1	5
IV02.dat	2	60	13/12/2008	1440	6	386.53	315018	815	5124	783	29	2	1	3
IV02.dat	2	60	14/12/2008	729	0	505.67	30340	60	1957	58	2	0	0	0
IV03.dat	3	60	11/01/2009	810	0	466.45	235093	504	4682	468	33	3	0	3
IV03.dat	3	60	12/01/2009	1440	1	382.56	262433	686	4415	637	48	1	0	1
IV03.dat	3	60	13/01/2009	1440	2	387.08	255859	661	7024	618	34	8	1	9
IV03.dat	3	60	14/01/2009	1440	3	306.17	210644	688	3378	667	21	0	0	0
IV03.dat	3	60	15/01/2009	1440	4	435.30	302533	695	5229	647	42	5	1	6
IV03.dat	3	60	16/01/2009	1440	5	388.70	253046	651	4836	615	32	4	0	4
IV03.dat	3	60	17/01/2009	1440	6	298.80	157767	528	6498	513	9	4	2	6
IV03.dat	3	60	18/01/2009	555	0	490.61	40721	83	2732	79	4	0	0	0

Abbreviations and meanings

R Output	***Meaning***
File	*File name of subject inserted*
ID	*Each subject has ID numbr, starting at number 1*
Epoch	*Length of epoch recorded*
Length	*Total number of minutes of measurement period, (1440 = 24 hours)*
Period	*Date of accelerometer recording*
Wkdy	*Each number corresponds to a certain day of the week, 0-Sunday, 1-Monday, 2-Wednesday…etc*
avg.cpm	*Average counts per minute*
tot.cnts	*Refers to the total number of counts measured in that time period*
val.time	*Refers to the number of minutes of recorded counts, after the removal of consecutive zeros*
permax	*Maximum counts*
Sirard.Sed	*Number of minutes spent in sedentary activity threshold, according to 'Sirard' cut-point*
Sirard.Light	*Number of minutes spent in light activity threshold, according to 'Sirard' cut-point*
Sirard.Mod	*Number of minutes spent in moderate activity threshold, according to 'Sirard' cut-point*
Sirard.Vig	*Number of minutes spent in vigorous activity threshold, according to 'Sirard' cut-point*
Sirard.MVPA	*Number of minutes spent in moderate to vigorours physical activity (MVPA) threshold, according to 'Sirard' cut-point*

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