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Breaks in Sedentary time in Young Children - Measures and Methodological Issues

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Author's Declaration

I declare that this thesis is the result of my own work, except where otherwise acknowledged, and has not been submitted for a higher degree to any other university or institution.

Zubaida Ibrahim .A. Alghaeed

Supervisor's Declaration

I certify that the work reported in this thesis has been performed by Zubaida Ibrahim .A. Alghaeed and that during the period of study she has fulfilled the conditions of the ordinances and regulations governing the Degree of Doctor of Medicine.

James Y. Paton

Disclosure Statement

I hereby declare that all of the research work for this thesis was undertaken by myself with the exception of:

> Data collection for Chapter 3 (Study 1b) and Chapter 4 (Study 2) were collected and provided by Dr Gwyneth Davies and Anne Martin, who were previous MSc students. The data of Study 1b was also used in Study 4 where it was merged with the data collected by myself. In all cases, the raw data was reanalysed by myself.

No writing assistance was utilized in the production of this thesis.

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Abstract

There is evidence that sedentary behaviour and breaks in sedentary time, independent of physical activity levels, influence human health.

In order to explore this relationship, accurate and validated measurement instruments are required. Such tools are also required for exploring the effects of factors such as the differences in sedentary behaviour between groups, e.g. overweight/obese vs. healthy weight children.

One promising instrument for making such measurements is the activPAL™ monitor. This is an event-based accelerometer with inbuilt inclinometer, and it may be more accurate for the measurement of sitting time and breaks in sitting than earlier instruments. An important setting in the monitor depends on the time required to define a new posture – the minimum sitting/upright period (MSUP). At present, the optimum activPAL™ MSUP setting is not known, particularly for children, who are likely to change posture faster than adults.

This thesis includes four studies:

Study 1 (Chapter 3): Using the activPAL™, we investigated the effect of variations in MSUP on total sitting time and breaks in sitting. **Methods:** *Study 1a:* In this in vitro experiment, the activPAL™ monitor was turned from a horizontal position to a vertical position manually (by hand) to simulate 5 sitting bouts. The length of the sitting time was varied from 1s to 10s. The number of the true events (i.e. 50) and the data from the activPAL™ (the number of sitting bouts for each bout length) was compared and represented in a graph. *Study 1b:* in data collected from children in a free-living environment (23 children (mean (SD) age 4.5yrs (0.7)) who wore the activPAL™ (24 hr/d) for 5-7d), we varied the setting of the MSUP. For each child, we calculated the following measures of sitting behaviour: volume (total time in sit/lie postures); number of breaks (number of sit/lie to stand transitions); number of sitting bouts (number of discrete periods spent sitting/lying); and pattern of accumulation of sitting (represented by accumulation curves and fragmentation index). We first studied the activPAL™ using the default setting of 10s MSUP, and then reduced this to 5s, 2s, and 1s.

Results:

Study 1a: the analysis software did not count sitting bouts of a shorter duration than the user defined MSUP in the new posture. For example, the sitting bouts with a period less than 10s were not counted when we used the activPAL™ setting of 10s MSUP. *Study 1b*: Comparing settings of 10, 5, 2, and 1s, there were no significant differences in total sitting time (6.2 hr (1.0), 6.3 hr (1.0), 6.4 hr (1.0), and 6.3 hr (1.6), respectively) between settings, but there were significant increases in: the apparent number of breaks - 8(3), 14(2), 21(4), and 28 (6)/hr) total number of bouts (118(18) vs 382 (80)); and Fragmentation Index (19.3 (3.7) vs 61.6 (16.4)), with a reduction in 50% bout length from 80s (14.7) to 42s (7.7) at 10s and 1s setting, respectively. **Conclusion:** With the activPAL™, breaks in sitting, but not total sitting time, are highly sensitive to the setting of the MSUP. Additional studies will be required to confirm these findings and to define the most appropriate MSUP for different age groups. Simple measures can characterise sitting behaviour in young children using the activPAL™.

Study 2 (Chapter 4): Posture transitions are likely to be much more rapid in young children than in adults. We investigated the optimum activPAL™ setting of MSUP to define a change in posture for measurement of sitting time and breaks in sitting (not previously known). We evaluated the validity of different minimum event duration settings against direct observation as the criterion method. **Methods:** In a convenience sample of 30 pre-school children (mean age 4.1yrs (SD 0.5)), we validated the activPAL™ measures of sitting time and breaks in sitting at different MSUP settings against direct observation. **Results:** In comparison with direct observation, a 2s setting had the smallest error relative to direct observation (95% limits of agreement: -14 to +17 sitting bouts/hr, mean difference 1.83, p = 0.2). **Conclusion:** For pre-school children, 2s appears to be an appropriate MSUP to define breaks in sitting using the activPAL™.

Study 3 (Chapter 5): The identification of risk factors for obesity is considered key to obesity prevention. Differences in time sitting compared to standing have been observed in obese and non-obese adults. Whether such differences are present between obese and non-obese children has not yet been examined. In a pilot study, we investigated differences in sitting behaviour between overweight/obese and healthy weight children. **Methods:** Overweight/obese children were recruited from weight management and dietetic clinics, the Active

Children Eating Smart programme, and from three primary schools, while healthy weight children were recruited from schools. The participants wore the activPAL™ (24 hr/d) for 5-7d. During waking time, the time spent sitting, number of sitting bouts, the 50% and 90% sitting bouts length, and the Fragmentation Index were measured in both groups using the activPAL™ with a 2s MSUP setting. Results were available for 26 healthy weight children (mean age 6.4yrs (SD 0.9), median BMI Z-score 0.04 (range -3.24 - 0.66)) and 13 overweight/obese children (mean age 6.4 yrs (SD 0.9), median BMI Z-score of 1.38 (range 1.14 - 3.10). **Results:** In healthy weight children during the waking hours, the mean (SD) percentage of waking time spent sitting was 53.0 % (6.4) representing 6.8 hrs (0.9) per day; mean (SD) total number of sitting bouts per day was 280 (65). The median (range) of 50% and 90% of sitting bouts were ≤50.0s (40.0-50.0) and ≤3.5 min (2.0-6.0), respectively. The mean (SD) Fragmentation Index was 42.1(12.7). In the 13 overweight/obese children, the mean (SD) percentage of waking time spent sitting was 52.4% (5.2), representing 6.9 hrs per day (SD 0.8). The total number of sitting bouts was 284 per day (66). The median (range) of 50% and 90% of sitting bouts length were ≤50.0s (40.0-50.0) and ≤3.5 min (3.0-6.0), respectively. The mean (SD) Fragmentation Index was 41.5 (9.6). **Conclusion:** Both healthy weight children and overweight/obese children in this study spent the majority of their waking time sitting. Furthermore, there were no significant difference in the sitting time, number, or duration of sitting bouts and the Fragmentation Index between the two groups in this study. **Study 4 (Chapter 6):** Previous studies have shown differences in the total sedentary time between boys and girls using accelerometers where sedentary behaviour was defined as low movement or low energy expenditure. In the present study, we examined whether there were also differences in breaks in sitting time. **Methods:** A convenience sample of 62 (32 girls: 30 boys) free-living healthy children (mean age 5.8yrs (SD1.3)) was recruited from nurseries and schools in Glasgow and Edinburgh, Scotland, who each wore the activPAL™ monitor continuously for 5–7 days. For each child, the components of sedentary behaviour were measured. **Results:** The percentage of waking time spent sitting was significantly higher in girls, the mean (SD) (54.4% (5.6)) compared to boys (50.9 (5.6)), (2-sample t-test, p-value <0.02). The total sitting time in girls vs. boys respectively per day was 6.9 (0.8) vs. (6.5) (0.9), (p-value <0.08). There were no significant differences in the number of sitting bouts, Fragmentation Index, or in 50% and

90% sitting bout length between girls and boys. **Conclusion:** This study suggested that girls spend more time in sitting than boys. However, there were no significant gender differences in the number or duration of sitting bouts.

Summary Conclusion

These studies have validated the activPAL™ as an instrument for measuring breaks in sedentary time in young children, and have established appropriate settings for making accurate measurements in this age group. Using these monitors to explore differences between obese and non-obese children, and between boys and girls, we found that, while there were differences between boys and girls in the total sitting time, there were no differences in the number of breaks between girls and boys, or between obese and non-obese children. These studies suggest that the activPAL™ may be useful to identify between group differences in sitting time and sitting fragmentation in future studies.

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Dedications

This thesis is dedicated to my husband Adel and children Mohamed, Ayoub and Hafed who have been very helpful and patient during this research work.

Publications

Paper

Alghaeed Z, Reilly JJ, Chastin SFM, Martin A, Davies G, Paton JY. The Influence of Minimum Sitting Period of the ActivPAL™ on the Measurement of Breaks in Sitting in Young Children. PLoS ONE 2013 Aug 14;8(8):e71854. (Appendix F)

Poster

The Frequency of the Posture Transitions and Sedentary Behaviours in Preschool Children. ICAMPAM conference. Glasgow, UK. 2011

The Influence of Minimum Sitting Period of the ActivPAL™ on the Measurement of Breaks in Sitting in Young Children. ICAMPAM conference. Amherst, Massachusetts, USA. 2013

Abbreviations

ACES   Active Children Eating Smart programme

BMI   Body Mass Index

CHD   Coronary Heart Disease

cpm   counts per minute

DLW   Doubly Labelled Water

HDL   High Density Lipoproteins

METs   Metabolic equivalent units

MSUP   Minimum Sitting/Upright Period

MVPA   Moderate to Vigorous intensity Physical Activity

NEAT   Non-exercise activity thermogenesis

SB   Sedentary Behaviour

SBRN   Sedentary Behaviour Research Network

SD   Sedentary Behaviour

SDS   Standard Deviation Score

ST   Sitting Time

TEE   Total Energy Expenditure

UK   United Kingdom

WC   Waist Circumference

WHO   World Health Organization

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Chapter 1

Introduction

1.1 Background

In the 1950s, Morris et al. (1) reported that individuals in physically active jobs had less coronary artery disease than those whose jobs involved sitting for prolonged periods. Bus conductors and postmen, both physically active workers, had a lower risk of coronary heart disease (CHD) than bus drivers and office workers, whose work was sedentary (1). This was one of the first studies to provide unequivocal evidence of the benefits of exercise on cardiovascular diseases in adults. Since then, studies have continued to define and evaluate the health benefits of physical activity in adults.

Physical activity has been defined as "any bodily movement produced by skeletal muscles that results in energy expenditure" (2). There is now substantial evidence that physical activity (particularly of moderate to vigorous intensity, MVPA) can play a significant preventative role in a number of important and prevalent contemporary diseases, including obesity, Type 2 diabetes, cardiovascular disease, some cancers, and with all-cause mortality in adults (3;4).

Children and young people also benefit from physical activity. Studies in children and young people show significant health benefits, such as improved cardio-metabolic profile, and increased bone mineral density, as well as mental health benefits with reduced rates of depression (5;6). More recent studies have even noted that regular physical activity may improve academic achievement (7;8).

Even pre-school children may benefit. Regular physical activity in this age group is associated with decreased adiposity, and improved measures of motor skill development, enhanced bone and muscle development, as well as improved psychosocial and cardio-metabolic health (9;10). It has also been shown that levels of physical activity track from early childhood, through adolescence and then into adulthood (11-13). Therefore, low physical activity in childhood may lead on to low adulthood physical activity.

Thus low physical activity in childhood may have two longer term effects on physical activity and health in adulthood: first, low physical activity may have direct effects on a child's health, effects which may be cumulative over time.

Secondly, there may be more indirect effects where a low level of physical activity during childhood tracks into adolescence and then adult life contributing to health problems as an adult (14). Since physical activity contributes to long-term cardiovascular health, it can be considered an essential preventive factor against cardiovascular diseases e.g. plasma lipoprotein levels, hypertension (15-17).

Figure 1.1 shows a summary model of direct and indirect effects of low physical activity in adolescence on adult health (17).
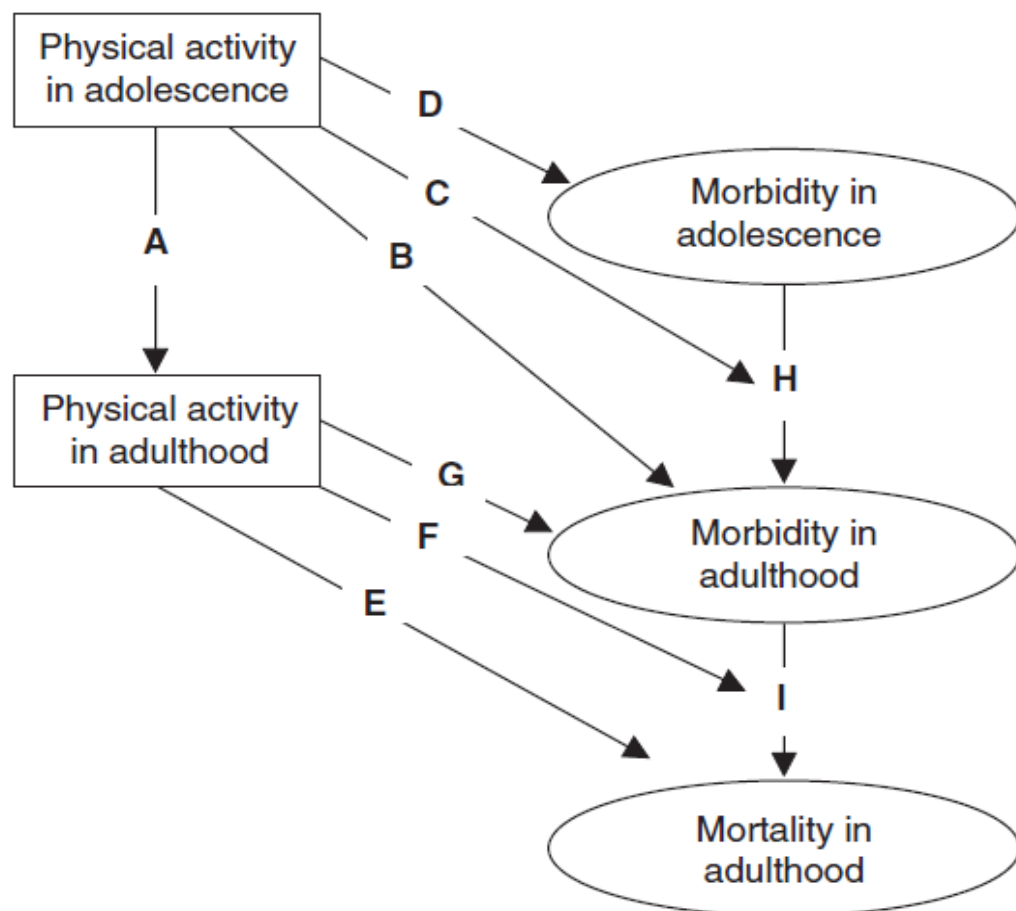
Figure 1.1 The association between adolescent physical activity and health: possible pathways. The proposed mechanisms include four direct effects (pathways A–D) and three indirect effects (pathways E–G). Pathway A – tracking of physical activity from adolescence to adulthood; B – direct influence of adolescent physical activity on adult morbidity; C – role of physical activity in treating adolescent morbidity; and D – short-term benefits of physical activity in adolescence on health. Other pathways (E–G) linking physical activity in adults to morbidity, prognosis and mortality, or that reflect progression from adolescent morbidity to adult morbidity (pathway H) or from the latter to mortality (pathway I). Adopted from reference (17)

The available evidence about physical activity from epidemiological surveys and intervention studies has been brought together into guidelines about how much activity the population at large should undertake to maintain and promote health. For example, the World Health Organization (WHO) guidelines (18), and the US Federal Physical Activity Guidelines (19), both recommend that adults take 30 min of moderate activity 5 days per week, and/or 20 min of vigorous activity 3 days per week (20).

Recommendations have also been developed for physical activity in children and young. For children and young people, the minimal physical activity target is for 60 min MVPA daily (5;18). For preschool children, a 2011 UK (21), physical activity guideline recommended 3 hrs every day of total volume of physical activity, not specifically MVPA. This is the same as the amount of physical activity recommended in recent Australian (22) and Canadian guidelines (10).

Despite recognised benefits and clear guideline recommendations, recent studies suggest that only a small percentage of the adult population actually meets the amount of physical activity recommended in physical activity guidelines (23-27). Compliance with the physical activity guidelines has also been evaluated in several studies into children, with the majority of children and adolescents failing to meet the physical activity guidelines in most European countries (28-32). Even in pre-school children, studies have reported that only a small proportion of time is spent being active, and many pre-school children also probably fail to meet current physical activity recommendations (33;34).

Thus the evidence suggests that the amount of physical activity undertaken across the age spectrum is low and well below what is recommended to maintain benefit from the benefits to health that exercise can bring. Aspects of modern life such as the development of the motor car, television and modern computers have meant that most people in society have become much less physically active and increasingly sedentary (35-37).

Recent years have seen a shift in focus from activity towards a growing recognition of an interest in the potentially harmful effects of this increasingly sedentary life style. It has become more clearly recognised that even though an individual meets the minimum daily physical activity guidelines, if they are sedentary for prolonged periods, they may be at an increased risk of certain diseases (35;37).

Research studies on sedentary behaviour and health are now proliferating (35;36), and have provided evidence relating prolonged sitting (sedentary behaviour) to poor health in adults (35;37), as well as children and adolescents (38;39). To date, the adverse effects of sedentary behaviour have not been well-established in preschool children (40).

Sedentary behaviour (SB) is now defined as "… any waking behaviour characterized by an energy expenditure ≤1.5 metabolic equivalent units (METs) while in a sitting or reclining posture" according to the Sedentary Behaviour Research Network (SBRN) (41).

At first studies, documented the length of time spent sitting. However, more more recently, the health benefits of breaks in SB (e.g. intermittently standing up) have been demonstrated in adults (42). At present, the amount of research into breaks in SB, which is the subject of this present thesis, is still limited, especially in the paediatric age group.

This introductory chapter brings together information about studies in physical activity and research developments in the study of SB and breaks in SB. It begins by briefly reviewing energy expenditure and activity and the methods used to measure activity. It then goes on to discuss sedentary behaviour and breaks in SB in more detail.

1.2 Physical Activity and Energy Expenditure

As noted, physical activity is defined as "any bodily movement produced by skeletal muscles that results in energy expenditure" (2). Vanhees et al. (43) showed that energy expenditure relates directly to the muscle mass used. The total energy expenditure (TEE) is usually considered to consist of a number of components:

A) Resting energy expenditure - this typically represents 60%-70% of TEE in low active populations, and is the energy needed at rest to maintain involuntary muscle contraction, maintain body temperature, and other basic autonomic functions.

B) Diet-induced energy expenditure - this is about 10% of TEE, and is the energy that needs to be expended to digest, absorb, and transport food and nutrients.

C) Energy expenditure due to physical activity (activity thermogenesis) - this represents about 20%-30% of TEE in low active populations. The exact amount of energy expended depends on the individual's activity (activity thermogenesis) (43).

D) Non-exercise activity thermogenesis (NEAT) – Levine et al. (44) were amongst the first to suggest that energy expenditure due to physical activity could be subdivided into energy expended on physical activity (exercise thermogenesis) and non-exercise activity thermogenesis (NEAT). NEAT includes daily living activities other than exercise: e.g. these encompass daily life behaviours such as posture variations (sitting or standing) and walking (45). NEAT is most variable in individuals who do not participate in regular physical activity (44).

It should be noted that energy expenditure on activities is often described in terms of METS where 'One MET is the energy cost of resting quietly, often defined in terms of oxygen uptake as 3.5 mL·kg-1·min-1 in adults.'(46). An energy expenditure ≤1.5 METs referred to sedentary (41), activities between 1.5 to 2.9 METS are usually referred to as light intensity, and greater than or equal to 3 METS as moderate to vigorous physical activity (MVPA)(47). In children, MET values are far higher but the concept can still be useful if one considers it in

terms of multiples of the individual's resting energy expenditure (REE), either measured or estimated (48).

1.2.1 Measurement of Physical Activity

The development of accurate and objective measurements of physical activity has been essential to research evaluating levels of physical activity and for studies determining the effectiveness of physical activity intervention programmes (43;49).The measures   of activity used in gathering the evidence about health benefits have employed a number of approaches, which can be broadly group into one of three categories (43). These methods are reviewed with methods used for measuring sedentary behaviour – which often employ similar conceptual approaches measuring instruments - considered later.

1.2.1.1 Criterion methods:

The main criterion methods used for physical activity research have been doubly labelled water (DLW), indirect calorimetry, and direct observation (gold standard).

DLW is performed in free-living conditions over a period of between 5 and 20 days. A dose of two non-radioactive-labelled isotopes is administered orally, and then a few blood, saliva, or urine samples are collected over the following 2 weeks. Measurement of the isotopes in the samples provides a measure of the CO_2 production, and, therefore, of energy expenditure. This method provides accurate measurements of total energy expenditure in free-living conditions.

DLW has some important limitations: it can only measure the total energy expenditure (TEE); the isotopes are difficult to obtain and very expensive; and it is not suitable for large studies. While the technique is applicable to children it is not commonly used in research in children because of the availability and cost of the doubly labelled water (43;49;50).

Indirect calorimetry measures EE from O_2 consumption and CO_2 production in a ventilated hood or in a respiration chamber. It is an accurate and valid measure. However, it is limited to laboratory experiments, and it has too many practical

problems to apply to large samples. This method will remain a good standard for the validation of other physical activity assessment methods (43;49;51),

Direct observation was one of the initial methods used to assess physical activity, and is often used to study the physical activity patterns of children. There are different observational systems available. Some of these systems are mentioned in Section 1.8.2.1 (43;49).

1.2.1.2 Other Objective methods

Several other objective techniques, such as heart rate monitoring, and particularly motion sensors (pedometers and accelerometers) are now widely available for the measurement of physical activity and have both limitations and advantages.

Heart rate monitoring depends on the linear relationship between heart rate and oxygen consumption (43). It provides an indirect assessment of the frequency, intensity, and duration of physical activity. Additionally, it is relatively inexpensive. However, heart rate is affected by environmental and emotional factors confounding the relation with intensity of exercise (43;49;52).

Pedometers are relatively simple and inexpensive electronic devices used to estimate the number of steps taken over a period of time. They calculate only steps and do not distinguish between different intensities of activity such as between walking and running (53).

Accelerometry is now widely used in physical activity research. This method uses accelerometers to measure acceleration produced by body movement. There are now a wide range of devices available with devices currently used in children including the Actigraph, currently one of the most commonly used; Actiwatch; Actical; and ActivTracer (43;54). The Actigraph and Actical are described in more detail in Section 1.8.2.2, as both of them have also been used in measurements of SB and breaks in SB, the main subject of this thesis.

1.2.1.3 Subjective methods:

These have included self-report questionnaires, interviewer-administered questionnaires, proxy-report questionnaires, reporting by another person (usually parents, carers or teachers), and diaries (55). Self-report questionnaires are the most common subjective method used to capture activity in adult studies e.g. the international physical activity questionnaire (IPAQ) and the physical activity diary (56;57).

Previous Day Physical Activity Recall (PDPAR) and the 3-Day Physical Activity Recall (3DPAR), a modification of PDPAR, have been widely used to assess physical activity in children and adolescences (56;58). However, the validity of self-report measures and interviewer-administered questionnaires has been low when used in children compared to adolescents. Studies involving children 10 years or younger should, therefore, rely on parental reports of child physical activity (proxy-report) with activity diaries being more suitable for adolescents and adults (43;49;56;59).

All these different questionnaire methods have been used to assess physical activity in research studies with many variations including: the length of questionnaires used (from short with 1–2 questions to more lengthy); the recall period with shorter periods, such as 1 day recall, being more valid and reliable than longer time periods (56;58).

Subjective methods provide inexpensive tools and are suitable for large samples. They may give useful information on the type, context, frequency and duration of activities. However, these methods have limitations, particularly recall and report biases and under-estimates or overestimation of levels of activity from participants or parents occur commonly. Furthermore they are vulnerable to influence by participants' interest (54;56;58;59). Children's activity is rapid and more interrupted than adult activity and therefore, may be more difficult to recall (60). The result is that questionnaire techniques are not suitable for young children particularly because of the developmental issues around recall and communication.

1.3 Sedentary Behaviour

Apart from his seminal observations on physical activity, Morris et al. also observed that the incidence of heart attacks was still high in adults free of clinical CHD who had sedentary desk job, even if they participated in leisure time physical activity (61). Morris's observation on the health risks of sedentary behaviour attracted much less attention. This started to change recently when Healy et al. (62) observed in 4,064 healthy, physically active adults (aged ≥ 25 yrs), who reported at least 150 min a week of MVPA, that television-viewing time was positively associated with a number of metabolic risk variables, including waist circumference (WC), systolic blood pressure, 2-hr plasma glucose, fasting plasma glucose, and triglycerides. The strongest associations with metabolic variables were observed in subjects with the highest amount of television viewing (>2.36 hrs per day) (62).

Recent research work (35;36) has increasingly focused on SB, and has shown that sedentary time, involving prolonged sitting time, is an independent risk factor for several health outcomes (35-37). At the same time there has been increasing appreciation of the fact that much of modern life is fundamentally sedentary. It has also become clear that not all SB is the same. Apart from the total time spent sitting, the occurrence and frequency of breaks in sedentary time may also have an influence on a person's health (47).

The following chapter outlines the definitions and terminology used in the literature about sedentary behaviour. Epidemiology, physiological mechanisms, and hazards linking SB and breaks in SB with poor metabolic health are also considered. Finally, the method of measurement of SB and breaks in SB are summarised.

1.3.1 What is Sedentary Behaviour?

The word 'sedentary' comes from the Latin word 'sedere', meaning "to sit" (36;63). Hamilton et al. (36) highlighted that the word sedentary now encompasses a sense of "lack of exercise", and is not limited to the original Latin meaning of sitting. This has led to standing being included with sitting in some studies. However, Owen et al. (47) commented that, according to the

results of a recent study (64), there is in fact light intensity activity performed in standing, which would previously have been considered sedentary. Thus, standing should probably not be classed as "a sedentary activity."

As noted above, the Sedentary Behaviour Research Network (SBRN) has proposed as a definition of sedentary behaviour "… any waking behaviour characterized by an energy expenditure ≤1.5 metabolic equivalent units (METs) while in a sitting or reclining posture" and this has become established as a consensus definition (41). Research has focused mainly on sitting (47) particularly activities such as TV viewing, computer use, and others with a low level of energy expenditure (less than 1.5 METs) rather than simply a lack of physical activity ('inactivity') (47). It should also be noted that screen time/TV viewing might have specific adverse health effects in children and adults (47;65-67). There has been some inconsistency in the literature in the use of the terms sitting and sedentary and widespread adoption of the SBRN definition of 'sedentary' would greatly improve the clarity of study related to these important health behaviours (41).

This shift in research focus from activity to sedentary and then sitting behaviour has been assisted by new opportunities to measure sitting behaviour directly and accurately provided by the most recent generation of accelerometers. The important technical developments have been the inclusion of inclinometers ,an example of such a device is the inclinometer-based activity monitor the "activPAL™" (PAL Technologies Ltd., Glasgow, UK) which can measure activity in the categories: sitting/lying, standing, and stepping (68). The activPAL™ monitor was the monitor used in the present studies, and it is described in detail in Section 1.8.2.2 and Section 2.2.

As noted above, SB includes a wide range of types of behaviour, such as watching television (TV), using a computer, sitting in motorised transportation, and workplace sitting (37;40;69). The majority of SB studies and guidelines focus mainly on screen time, such as TV viewing and using a computer, as common and important SBs (10;22;38;40;69;70).

In this thesis, we objectively measured the sitting time (ST) and activities that are spent sitting or lying down by using the activPAL™ and did not limit SB to screen-time. Therefore, the abbreviation "ST" will be used in our studies using

the activPAL™, in order to distinguish these activPAL™ studies from other studies.

1.3.2 Sedentary Behaviour Guidelines

As for physical activity, guidelines have been developed about sedentary behaviour. The Canadian Sedentary Behaviour Guidelines for Children (aged 5–11 yrs) and Youth (aged 12–17 yrs) (71), and the American Academy of Paediatrics for school-age children recommend no more than 2 hrs screen time per day for all school-age children (72). The Australian guidelines (22) and Canadian guidelines (10) for pre-schoolers recommend a restriction of screen based SB to <1 hr per day (10;22).

Almost all of the guidelines have focused on recommendations for children and adolescents; there appears to be little or no evidence to support the specific time recommendations of SB for health per day or week (21); adult guidelines have just included recommendations to "minimise the amount of time spent being sedentary (sitting) for extended periods" (21).

Again similar to activity guidelines, there is evidence that compliance with the Sedentary Behaviour Guidelines is poor. Some studies have estimated the proportion of children and adolescents that exceed hourly thresholds of television viewing (73-77). Reviewing these studies Pate et al. (69) described the levels and forms of SB in school-age children and adolescents and reported that they typically exceed 3 hrs of television viewing per day (69). In preschool children, evidence suggest that this age group typically spends between 1.8 and 3.3 hrs per day on screen time (40). Thus there is evidence that the majority of preschoolers, older children and adolescents already exceed the recommended amount of screen time (40;69).

1.4 Breaks in Sedentary Time

A cross-sectional study by Healy (42) first reported that increased breaks in sedentary time, resulting in shorter periods of uninterrupted sitting, were beneficially associated with WC and body mass index (BMI), triglycerides, and 2-hr plasma glucose. In the study, 168 adults (65 M, 103 F; mean age 53.4 yrs)

wore the Actigraph monitor during waking hours for seven days. Breaks were considered as any interruptions in sedentary time from a 'sedentary' (<100 counts per minute (cpm)) to an 'active' state (≥100 cpm). The beneficial relationships the authors observed with increasing breaks in sitting were independent of total sitting time. This important study has helped shift research towards how sedentary time is accumulated, rather than considering only the total volume of sedentary time.

Using the Actigraph monitor, Healy et al. (42) were the first to propose that a break was an interruption in sedentary time in which the accelerometer count was ≥100 (cpm) over a 60s epoch (Figure 1.2) (42). (The Actigraph is described in more detail in Sections 1.8.2.2 and 1.9.1.1.) Subsequently, researchers have used this same definition of breaks in sedentary time both in adult studies (78-84), and in studies of children and youth using the Actigraph and the Actical to measure breaks in sedentary time (85-95)(Table 1.2, Table 1.3). In studies using the activPAL™, breaks in sitting time have been defined as transitions from sit/lie to stand or step in both adult (96-100) and children studies (87;96-99;101-103), Table 1.1. (The activPAL™ is described in more detail in Sections 1.8.2. 2. and 2.2.)

Healy et al.'s findings were in keeping with previous observations in animal models (36;104). The animal studies compared the physiology of inactivity with the physiology of physical activity in laboratory rats. They reported the high density lipoprotein (HDL) cholesterol level to be 20% lower in the rats that had been prevented from standing (inactive rats), as compared with a normal standing/ambulatory group. The difference was evident within a day, and continued throughout 11 days. Detailed studies showed that there was a decrease in the lipoprotein lipase enzyme in the blood vessels of the postural support muscle (in the legs) of the rats that were not allowed to stand/ambulate. Lipoprotein Lipase enzyme captures triglyceride from the blood to be oxidised by muscle. The decrease of Lipoprotein Lipase enzyme during inactivity was much more than the increase after physical activity.

Taken together, this research suggests that there are important physiological differences between inactivity and physical activity, and also provides evidence that sitting and the lack of interruption in periods of sitting has adverse

physiological effects (36;104). These issues are dealt with in more detail in section 1.7 below.
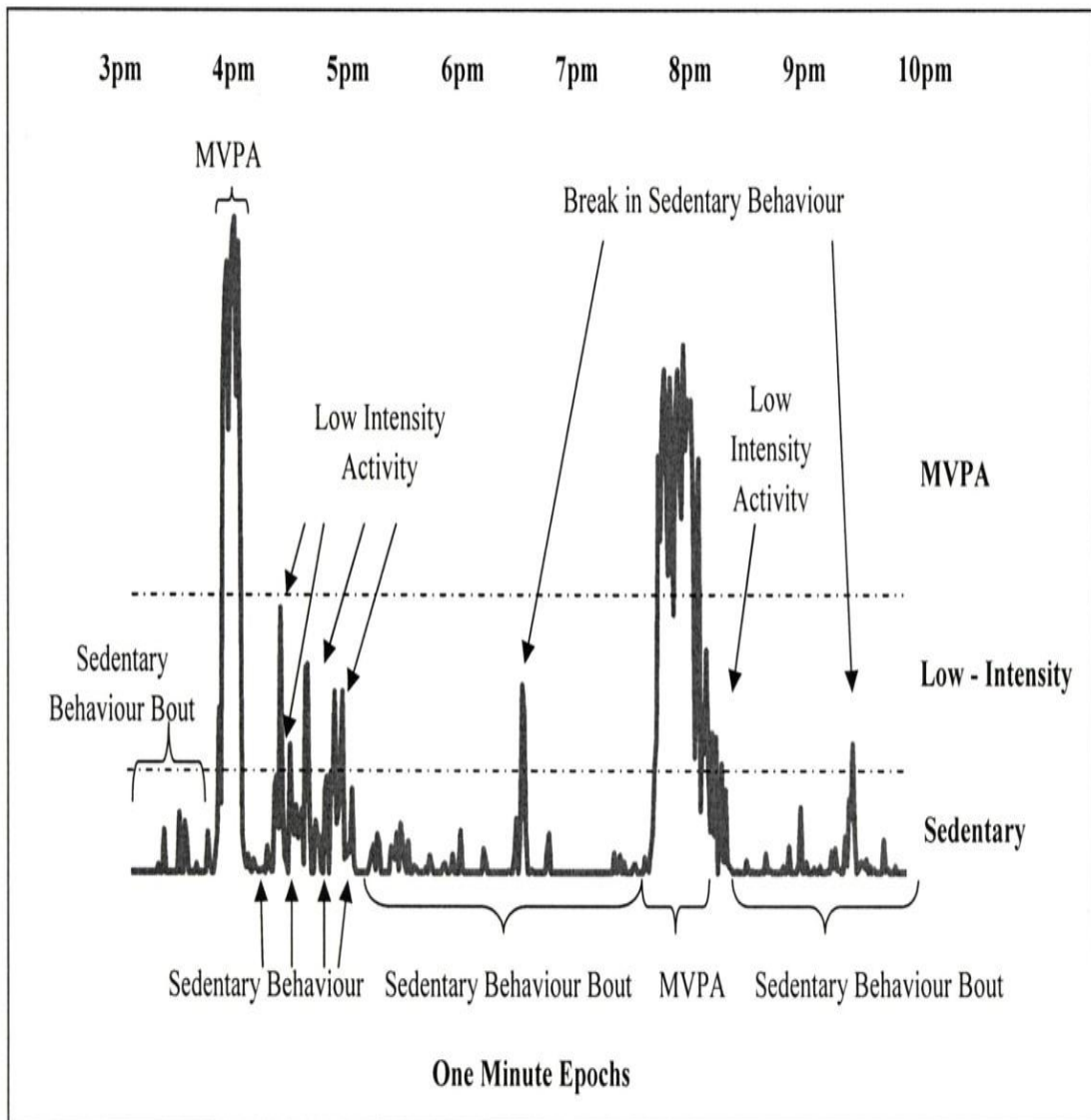
Figure 1.2 The x-axis represents 7 hours of time, in 1 min epoch values, of accelerometry measurements. The height of the data on the y-axis represents the intensity of the epoch values, with higher data points equalling higher intensities. Adopted from reference (105). Breaks were considered as any interruptions in sedentary time from a 'sedentary' (<100 cpm) to an 'active' state (≥100 cpm) using the actigraph.

1.5 Epidemiology of Sedentary Behaviours and Breaks in Sedentary Behaviours in Children and Adolescents

1.5.1 The Time Spent in Objectively Measured Sedentary Behaviour

As mentioned in Section 1.3.2, Pate et al. (69) noted that school age children and adolescents typically exceed 3 hrs of television viewing per day (69) while preschool children typically spend between 1.8 and 3.3 hrs per day on screen time (40). In these studies, the measures were obtained by subjective methods, such as parent-report and self-report (40;69). However, many studies using objective methods (such as accelerometry) to assess time in SB have also found that children and adolescents spend the majority of their time in SB. A recent systematic review by Hinkley et al. (40) concluded that the percentage of objectively measured SB accounted for between 50% and 80% of the total day in preschoolers (40). Further, in a large UK-wide representative sample (106), where the physical activity and sedentary time were objectively measured, using the Actigraph accelerometer (with sedentary time defined as <100 cpm) in 6,497 primary school aged children (mean age 7.5 yrs), Griffiths et al. found (106) that more than half of all children spent the majority of the day in sedentary behaviours, with on average of 6.4 hrs per day being spent sedentary (106).

The time spent in SB was also high among older children and youths in a prospective study of a large cohort in the UK by Mitchell et al. (107). Here, the objectively measured SB (using the Actigraph accelerometer, sedentary time defined as <200 cpm) measured 7.1 hrs/day at age 12 (n = 5,429), 8.0 hrs/day at 14 yrs (n = 3,486) and 8.6 hrs/day at 16 yrs (n = 1,971) (107).

Equivalent SB data for UK pre-schoolers were reported in a recent study by Hesketh et al (108) where the sedentary time was objectively measured (Combined heart-rate monitor and accelerometer) in 554 preschool children (mean age 4.1 y (0.1)). They found that these preschool children spent 4.7(1.6) hr/day in sedentary time (108).

Two recent North American studies also reported similar findings; a recent report (109) from the Canadian Health Measures Survey (CHMS), using a nationally representative sample of school-aged children and adolescents (aged

6 to 19 yrs), indicated that children and youth were sedentary for approximately 7.4 hrs per day (55% of waking hours), 8.8 hrs/day (64% of waking hours), and 9.4 hrs/day (69% of waking hours) for age 6 to 10 yrs, 11 to 14 yrs, and 15 to 19 yrs, respectively, using the Actical accelerometer with a sedentary cut-point of <100 cpm (109). Using data from the 2003-2004 National Health and Nutrition Examination survey in the USA, Matthews et al.(110), concluded that children and adults in the United States spend the majority of their waking day in SB (measured using the Actigraph accelerometer with a cut-point <100 cpm), for both sexes, with time spent sedentary in children aged 6–11 yrs and 12–15 yrs measured as 6.1 and 7.5 (0.1) hrs/day, respectively. Adults spend even more time in SB than children, spending 8 hrs/day (about 60% of their time) sedentary (110).

1.5.2 Sedentary Behaviour and Breaks in Sedentary Behaviour and Age

Some studies have investigated whether there is a change in SB with age (69;107;110;111). For example, a recent cohort study observed that sedentary time increased on average 2.45 hrs per day from childhood to adolescence. This was based on data from Actigraph accelerometer studies, with time spent in sedentary time defined as a cut point of ≤100 cpm (111). In a longitudinal study with SB measured daily and objectively, Mitchell et al. (92) (107), found an increase in SB of approximately 90 min per day from age 12 to 16 yrs (107). This was broadly in agreement with cross-sectional data from Matthews et al. (110) who found that SB increased about 2hrs/day from ages 6–11 yrs to ages 16–19 yrs (110).

To date, only one longitudinal study has described the changes in the frequency of sedentary breaks in relation to age. This study by Kwon et al. (95) noted that breaks in sedentary time (using the Actigraph with breaks defined as accelerometer counts ≥100 cpm for a 60s epoch) decreased by >200 breaks/day over a 10 yrs period, from ages 5 to 15 yrs (95).

1.5.3 Sedentary Behaviour and Breaks in Sedentary Behaviour and Gender Differences

Gender differences in objectively measured SB have been observed in some studies in children (69;107;110). These studies found that sedentary time was higher in girls than boys (using the Actigraph with SB defined as <100 cpm for a 60s epoch). The author is unaware of any studies on differences in TV viewing between boys and girls in early childhood, but, in adolescence, TV viewing greatly exceeded the 2hr/day recommended in many studies (112).

Interestingly, the Longitudinal Iowa Bone Development Study of Kwon et al. (88) found the frequency of sedentary breaks (Actigraph monitor with sedentary time broken if accelerometer counts were ≥100 cpm for a 60s epoch) was significantly (p <0.01) higher in boys than girls at age 11 yrs (n = 520), 13 yrs (n = 454) and 15 yrs (n = 344) (88). Up to now, this has been the only study that described the change in the frequency of sedentary breaks over a 10 year period from ages 5 to 15.

To date, no study has examined gender differences in sedentary time and breaks in sedentary behaviours in children using a tool designed to measure sitting time and breaks in sitting time directly (such as the activPAL™) to examine SB components without the use of thresholds. In this thesis, the aim of Chapter 6 was to undertake such a study, and compare the ST and breaks in ST in boys vs. girls in young children, as a way of determining if the activPAL™ would be useful for examining between-group differences.

1.5.4 Sedentary Behaviour and Socioeconomic Status

Pate et al. (69) recently reported that some studies found higher levels of SB, mainly screen-based SB, in children and adolescents from a lower socioeconomic status background, after lower income, lower level of parental education, and lower level of parental employment (69) had been accounted for. A review by Hinkley et al. (40), where the majority of the studies included in the review measured television viewing as their sedentary behavioural outcome, also found that socioeconomic status may be an important influence on SB in the preschool age. However, until now, there has not been enough evidence on the association

between young children's SB and socioeconomic status (40). There is also little or no evidence about breaks in sitting time as measured by the activPAL™.

1.6 Sedentary Behaviour and Breaks in Sedentary Behaviour and Health Outcomes

Some of the hazardous effects of SB and the benefit of the breaks in SB on individuals' and populations' health are described in brief below, as a full discussion is beyond the scope of this thesis.

1.6.1 Sedentary Behaviour and Breaks in Sedentary Behaviour and Obesity

The consequences of obesity are numerous. Obesity is associated with all-cause mortality in adults (113) and multiple co-morbidities, such as cardiovascular, metabolic, pulmonary, and psychological disorders in children and adolescents (114). Moreover, overweight children are more likely to become obese adults (115;116). Therefore, obesity is not simply a cosmetic issue, but is a genuine pathological state (114).

The aetiology and pathogenesis of obesity are extremely important in the search for understanding and finding a potential solution to this issue. Obesity occurs due to a chronic energy imbalance of excessive dietary intake alongside reduced energy expenditure. The treatment of obesity is conceptually simple, in that the imbalance between intake and output has to be prevented if obesity is to be prevented. As mentioned before, the NEAT activities of daily living, such as sitting (sedentary), standing, posture transitions, and walking, are one of the most variable components of activity energy expenditure, especially in individuals who do not participate in regular exercise (44). Therefore, both SB and, more recently, breaks in SB have been of great interest in obesity research. In the following section, studies that investigated the association between SB and breaks in SB and obesity will be described briefly.

1.6.1.1 In adults

Sedentary Behaviour and Obesity

In developed countries, more than 60% of middle-aged adult individuals are overweight or obese (42). Specific SB, such as television viewing, is known to increase the risk of obesity (62). TV viewing time has been associated with an increased risk of becoming overweight or obese in adult men and women, a risk that is independent of leisure time, physical activity, and diet (117).

A systematic review of 48 longitudinal studies published between 1996 and 2011 (in which only three studies measured sedentary time objectively) that summarised the associations between SB and all health outcomes in adults, observed that elevated levels of SB were consistently associated with weight gain in both sexes. However, additional evidence from longitudinal studies is required, especially in combination with objectively measured SB (47).

Breaks in Sedentary Behaviours and Obesity

Healy et al. (42), in a study described previously in Section 1.4, reported that, independent of total sedentary time and moderate-to-vigorous intensity activity, increased breaks in SB are beneficially associated with WC and BMI (p = 0.026) (42). Similarly, another cross-sectional study by Healy (83) observed that, independent of total sedentary time, breaks in SB were beneficially associated with WC ((p = 0.05) in 4,757 adults (mean age 46.5 yrs (SD 14.2)), 50% males)). Similarly, Cooper et al.(81) observed that breaks in sedentary time were associated with lower WC (p = 0.003) in 528 adults (mean age 59.8, (SD 10.0)) with Type 2 diabetes (81). Henson et al. (84) studied 878 participants (age range 32.9 to 63.7) with known risk factors for Type 2 diabetes mellitus, and also found that breaks in SB were significantly inversely associated with measures of adiposity (WC, p<0.001, BMI, p<0.01) (84). Bankoski et al (78), observed that higher sedentary time and fewer sedentary breaks were related to a significantly increased of WC (p <0.01). These studies are summarised in table 1.1 and table 1.2

In all the above studies ((78;81;83;84;118) table 1.1 and table 1.2), the participants wore the Actigraph during waking hours for up to seven days, and breaks were considered as any interruptions in sedentary time from a sedentary (<100 cpm) to an active state (≥100 cpm).

Actical data (119) (table 1.1) of 4935 adults aged 20-79 yrs were collected in the 2007/09 and 2009/11 Canadian Health Measures Survey, and total sedentary time (defined as < 100 cpm), patterns of sedentary time (≥20 minute prolonged sedentary bouts, number of sedentary breaks) were calculated. WC was measured. They observed, on average, each additional 10 breaks/day was associated with 0.83 cm lower WC.

An important caveat about these above studies is that the Actigraph and the Actical have yet not been established as valid tools to measure breaks in SB, and future studies need to confirm these results by using a validated tool, such as the activPAL™.

1.6.1.2 In Children and Young people

Childhood obesity has become a major public health epidemic, with the prevalence increasing worldwide (120). A recent systematic review by Rokholm et al. (121) showed that overweight and obesity prevalence in some childhood age groups might have been flattening or even decreasing over the last 10 yrs in Western countries, although the overall prevalence is still high (121). The WHO estimated that more than 40 million children under the age of five were overweight in 2011 (122).

Sedentary Behaviour and Obesity

Previous reviews (38;65;123-126) examined the association between sedentary behaviour and markers of cardio-metabolic risk in children and youth. Four reviews (38;65;123;124) had included both subjectively (mainly TV-viewing) and objectively measured SB, and one had only reviewed longitudinal studies (123).The other two reviews (125;126) included only objectively measured SB ,and one had reviewed only longitudinal studies (126). The results that were obtained from these reviews will be mentioned briefly.

Tremblay et al. (65) reported a systematic review of the relationship between SB and overweight/obesity in 199 cross-sectional studies (published from 1958 to 2009) in 691759 children and youths aged 5-17 yrs. Ninety-four cross-sectional studies (36 studies in the children's age group) found a positive association between SB and increased weight status (mainly TV-viewing; only one study objectively measured SB). Nineteen longitudinal studies reported that watching TV was associated with weight gain. They suggested that sedentary behaviours, mainly TV- viewing, related to an increased risk of being overweight or obese (65).

These findings have also been supported by evidence in a more recent systematic review by Mitchell and Byun (38). They summarised findings from cross-sectional and longitudinal studies, published between January 2008 and September 2012. These stduies investigated the association between sedentary behaviour (watching TV and objectively measured sedentary behaviour) and health outcomes in children and adolescents. The researchers' finding were from 43 cross-sectional studies of which 32 studies reported an association between SB (watching TV) and higher obesity in children and adolescents (sample size ranged from 72 to 54 863). Interestingly, the positive associations between SB (watching TV) and obesity remained in the 15 studies (sample size ranged from 153 to 18 784) that adjusted for MVPA (127-141). These results were confirmed in 8 longitudinal studies (sample size ranged from 465 to 7334) (142-149). In four studies that adjusted for MVPA (142;143;147;148) SB (watching TV) rather than activity was driving the association. Mitchell and Byun (38) also reviewed studies that investigated the association between objectively measured SB and obesity, from seven (sample size ranged from 53 to 20 871) objectively measured cross-sectional studies (150-156).No association was observed in four studies (150;152;155;156), even though one of these studies by Ekelund et al had the largest sample size and most robust study design (150). This last study pooled data from 14 studies between 1998 and 2009 comprising 20 871 children (aged 4-18 yrs) from the International Children's Accelerometry Database. Sedentary time was measured using Actigraph after reanalyzing raw data (time spent sedentary was defined as all minutes showing < 100 cpm). In this study Ekelund et al found that time spent sedentary was not related to adiposity (WC) or other

cardio-metabolic risk factors (fasting insulin, fasting triglycerides, HDL cholesterol and blood pressure) after adjusting for time spent in MVPA (150).

Mitchell and Byun (38) reported a positive association in only 3 cross-sectional studies (sample size ranged from 1458 to 5434) (151;153;154), but no association was observed after adjustment for MVPA in 2 studies (151;154). From 4 objectively measured longitudinal studies (157-160), there was one study by Mitchell et al. that adjusted for MVPA reported an association (158). This was a longitudinal study of the National Institute of Child Health and Human Development (NICHD) in the USA examining the relationship between objectively measured sedentary time (using ActiGraph and a cut point of <100 cpm defined sedentary behaviour) and BMI among 789 participants between 9 and 15 years of age. The participants wore the ActiGraph when aged 9, 11 and 12 years and again when aged 15 years. They found that time spent in sedentary behaviour was positively associated with an increase in BMI at the 50th, 75th and 90th percentiles between ages 9 and 15 years, independent of MVPA(158). Mitchell and Byun (38) concluded that the positive associations between SB and adiposity was obtained mainly from screen-based sedentary behaviour (mainly watching TV) studies. More evidence was necessary about the relationship betweenobesity and objectively measured SB.

A recent review by Ekelund et al. (124) examined the association between sedentary behaviour (watching TV and objectively measured sedentary behaviour) and adiposity with adjustment for objectively measured physical activity in children and adolescents aged 3 to 18yrs. The authors identified eight studies (mainly TV-viewing), (six cross-sectional (129;161-165) and two prospective studies) (166;167) of which four studies (129;161;162;167) (including one prospective study (167)) found a positive association between TV-viewing and adiposity. Six cross-sectional (150;151;154;155;168;169) and 3 prospective studies (95;158;159) that objectively measured SB were identified, and after adjustment for MVPA, the positive association between SB and adiposity was observed in only the one study by Mitchell et al, described above (158). This has led some to speculate that any apparent health 'effects' of SB may in fact be effects of low MVPA(124).

A systematic review by Chinapaw et al. (123) described the prospective relationship between sedentary behaviour and health indicators in children and adolescents. They included articles published from 1989 up to April 2010. Twenty-six studies examined the longitudinal relationship between SB and obesity (only one study measured sedentary time by accelerometers (157)),and nine studies observed a positive relationship (all studies looked at TV-viewing). However, the authors concluded that there was insufficient evidence for this positive longitudinal relationship, and further studies are needed(123).

Fröberg and Raustorp reviewed (125) studies that examined the association between objectively measured SB and markers of cardio-metabolic risk in youths. They included articles published between January 2000 and October 2013 (both cross-sectional and longitudinal studies). Forty-five studies (7 longitudinal studies) were included, with only three studies (94;158;170), including one longitudinal study (158), showing a positive association between SB and obesity. The association remained in the one study by Mitchell et al ,described above, that adjusted for MVPA (158).The authors concluded that there is no clear evidence to confirm that SB is associated with obesity, and future objectively measured sedentary behaviour studies that are adjusted for MVPA are warranted (125).

Another recent systematic review by Tanaka et al. (126) also examined the longitudinal relationships between objectively measured SB and adiposity; they included the studies published between the 1950s and November 2013. From 3 eligible papers(95;158;159), one study by Mitchell et al, described above (158) found a positive association between increased SB and greater increases in BMI after adjusting for MVPA. The authors found insufficient evidence, and suggested that more evidence was necessary to establish if sedentary time was associated with adiposity in childhood and adolescence (126).

In conclusion, some studies, mainly cross sectional rather than prospective studies, have found that objectively measured sedentary time does not seem to be associated with poor health outcomes in children and adolescents when MVPA is adjusted for i.e., the association is with MVPA, not sedentary time. Hence the precise role of objectively measured sedentary time in influencing health in children and adolescents is currently unclear and is being debated. In particular,

watching TV appears to be more strongly associated with obesity and cardiometabolic risk factors than the total sedentary time assessed by accelerometers in children and adolescence (38;65;123-126;171).

SB, particularly television-viewing, is also one of the risk factors of overweight/obesity in preschool children (172). However, there is much less evidence in this age group than in older children, and, thus, further studies are needed (40).

While screen time (predominantly TV viewing) is a sedentary activity, some studies have shown that more food is consumed while watching TV, especially unhealthy and advertised food and drink such as pizza, snack foods, and soda, particularly by children and adolescents, and all of these are thought to affect their weight (127;173-176).The association is drawn mainly from cross-sectional studies and more longitudinal studies examining the association between the amount and the type of food eaten while watching TV and obesity are needed (177). However, there are also some studies have observed that the association between more TV-viewing and obesity was independent of food intake while watching TV so the precise pathophysiological explanation is not clear (127;128;142).

The issue of bi-directional causation of obesity

Both insufficient physical activity and excessive SB have been suggested as possible causes of obesity. However, the relationship between them may be bi-directional: low physical activity and excessive SB may predispose to higher fatness while also high fatness may can predispose to lower physical activity and higher level of SB, in adult (178), in adolescents and in children (150;179-181). Only a small number of studies to date (178-181) have assessed the possible effects of obesity on physical activity. Using data from the International Children's Accelerometry Database Ekelund et al. (150) examined prospective associations between objectively measured physical activity, SB (using Actigraph (GT1M) and the sedentary time defined as less than 100 cpm), and WC in 6413 children and adolescents (aged 4-18 years). Study participants were followed up for 2 years and the measurements were available at two points. The authors observed that a higher baseline WC was associated with increased time spent

sedentary, but the baseline WC was not associated with time in physical activity at the follow-up. The authors suggested that the association between physical activity, sedentary time, and weight gain may be bidirectional (150).

Until now, few authors have explored the possibility of a bidirectional association. Further longitudinal studies starting at a young age, using valid measurement methods and controlling for relevant confounding factors, such as dietary intake, will be required to clarify this issue of bidirectional causation (124).

Breaks in Sedentary Time and Obesity

As mentioned above, while a benefit of breaks in sedentary time has been observed in adults, these findings have yet to be replicated in children and young people. To our knowledge, there eight studies (86;87;89-94) (Table 1.4, Table 1.5) that have examined the association between breaks in sedentary time and health indicators in children and youths, with only two cross-sectional studies in Canada finding a positive association (90;91). The results from the studies (Saunders et al (90); Colley et al. (91)) are summarised in Table 1.4 and Table 1.5. Saunders et al. (90) observed that the breaks in sedentary time were independently and positively associated with lower BMI Z-score in both sexes. The number of sedentary bouts lasting 5–9 min was negatively associated with WC in girls only, while the number of bouts lasting 10–14 min was positively associated with BMI Z-score in boys (all $p < 0.05$). This study was conducted in a group of 522 children, with a mean age of 9.2 (range 8.0–11.0 yrs), who had a family history of obesity (with at least one biological parent with obesity), with each wearing an Actigraph during waking hours for seven days (90). Likewise, in 1,608 children and youths between the ages of 6.0 and 19.0 yrs, Colley et al. (91) found that prolonged bouts of SB measured using the Actical were positively associated with BMI and WC, independent of MVPA, in boys aged 11-14 yrs. However, these associations were not observed in older or younger boys, or in girls of any age. The above studies used the Actigraph or Actical to measure breaks in sedentary time. Neither accelerometer has been validated for measuring breaks in sedentary time (91). SB measurements are discussed in Section 1.8.

1.6.2 Sedentary Behaviour and Breaks in Sedentary Time and Other Cardio-metabolic Risk Factors

Cardio-metabolic risk factors, such as obesity, hypertension, the dys-lipidaemic combination of low levels of high density lipoprotein cholesterol and high levels of triglyceride, and impaired glucose tolerance, are known predictors of coronary heart disease and Type 2 diabetes in adults (105).

1.6.2.1 In Adults

Sedentary Behaviour and Other Cardio-metabolic Risk Factors

A meta-analysis of 10 cross-sectional studies in which only one study used objectively measured sedentary time in 21,393 adults (≥18 yrs of age) examined SB and metabolic risk factors. The study concluded that prolonged time spent in SB was associated with a 73% increase in metabolic syndrome risk. Longitudinal studies are considered necessary to explain and confirm this relationship (182). At present, the available evidence from longitudinal studies (mentioned in Section 1.7.1.1) concluded that there was at present inadequate evidence to conclude that a longitudinal relationship exists between SB and markers of cardio-metabolic health (47).

Breaks in Sedentary Time and Other Cardio-metabolic Risk Factors

The cross-sectional study by Healy (42) (described in Section 1.6.1.1) also observed that increased breaks in sedentary time were beneficially associated with other metabolic risk variables, particularly triglycerides, and 2-h plasma glucose. Carson et al (119)(table1.1) found that total sedentary time and time in ≥20 mins prolonged sedentary bouts were associated with higher insulin and lower diastolic blood pressure levels (P < 0.05) while increasing sedentary breaks by 10 per day was associated with 0.32 mmHg lower systolic blood pressure, 0.01 mmol/l higher HDL-cholesterol, 3.72 % lower triglycerides, 0.57 % lower glucose, and 4.19 % lower insulin in a large representative sample of Canadian adults (119).The relation between breaks and other metabolic risks has been noted in other studies (table 1.2) One study conducted in the general population (83) found that breaks in sedentary time were beneficially associated with C-reactive protein, and fasting plasma glucose (83). The other study (665 adults with metabolic syndrome compared with 702 adults without metabolic

syndrome) concluded that more sedentary time and less breaks in sedentary time were related to a significantly increase of triglyceride, fasting glucose and blood pressure (p <0.01 for all) (78).

Nevertheless, the relation between breaks and other metabolic risks was not confirmed by other studies conducted in those with Type 2 diabetes (81), and populations with known risk factors for Type 2 diabetes mellitus (84).

1.6.2.2 In Children and adolescents

Sedentary Behaviour and Other Cardio-metabolic Risk Factors

Systematic reviews (38;39;65;123;125) in children and adolescents concluded that there is a positive association between SB (mainly TV-viewing) and other cardio-metabolic risk factors, although in most objectively measured studies the associations of SB and health disappear after adjustment for MVPA. At present, the evidence base is still limited, and further studies, especially objectively measured studies, are needed to confirm the presence and strength of association (38;39;65;123;125;183).

Breaks in Sedentary and Other Cardio-metabolic Risk Factors

To date, there has also been little research investigating breaks in SB in relation to cardio-metabolic risk factors, with only five cross-sectional studies (86;90-92;94), in Table 1.3 and Table 1.4, into children and adolescents, and no work on young children. From the three available studies, only the study by Saunders et al. (90) (described in Section 1.6.1.2 and in Table 1.4) observed that breaks in SB are independently associated with markers of cardio-metabolic risk, including fasting insulin, fasting glucose, triglycerides, and HDL-cholesterol in children with a family history of obesity (90). In other four studies (86;91;92;94), this association was not observed.

Table 1.1 The associations between objectively measured sedentary time and breaks in sedentary time and cardiometabolic health in adults

Study	Study group	Monitor	Breaks	Duration	Health outcome	Adjusted PA	Conclusion
Carson et al. (119) 2014 Canada Cross-sectional Study	Full sample 4935 aged 45.9 yrs(15.1) Fasting sub-sample 2551 aged 46.4 (15.3) (participants who provided fasting blood measures)	Actical	Sedentary time broken if accelerometer counts were ≥100 cpm for 60s epoch	4 days	WC, systolic and diastolic blood pressure, HDL cholesterol and C - reactive protein were measured in the full sample.Triglycerides, LDL cholesterol, glucose, and insulin were measured in a sub-sample	Yes	Total sedentary time and time in≥20 minute prolonged sedentary bouts were **associated** with higher insulin and lower diastolic blood pressure levels. Each additional **10 breaks/day** was associated with lower WC, lower systolic blood pressure, higher HDL-cholesterol, lower triglycerides, lower glucose, and lower insulin.
Henson et al. (84) 2013 UK Cross-sectional Study	878 adults aged 48.3 yrs (6.7)	ActiGraph GT3X	A break was considered as any interruption in sedentary time (≥25 counts per 15 s)	4 days	fasting plasma glucose, 2 h plasma glucose total cholesterol, HDL-cholesterol and triacylglycerol.	Yes	Sedentary time was associated with 2 h glucose, triacylglycerol and HDL-cholesterol.Breaks in sedentary time was significantly inversely associated with measures of adiposity.
Cooper et al. (81) 2012 UK Cross-sectional and Longitudinal Study	528 adults (newly diagnosed type 2 diabetes) aged 59.8 yrs (10.0)	ActiGraph	Sedentary time broken if accelerometer counts were ≥100 cpm for 60s epoch	5-7 days	WC, HDL-cholesterol, insulin and glucose levels and HOMA of insulin resistance were measured at baseline and at 6 months follow-up	Yes	In cross-sectional analyses; higher sedentary time was associated with a larger WC, higher insulin and lower HDL-cholesterol. More breaks were associated with lower WC. In longitudinal analyses; sedentary time was less strongly associated with WC than at baseline, while associations with HDL-cholesterol were similar. The breaks were more strongly associated with WC.

Table 1.2 (Continued) The associations between objectively measured sedentary time and breaks in sedentary time and cardiometabolic health in adults

Study	Study group	Monitor	Breaks	Duration	Health outcome	Adjusted PA	Conclusion
Healy et al. (83) 2011 USA Cross-sectional Study	4757 adults aged 46.5 yrs (14.2)	Actigraph	Sedentary time broken if accelerometer counts were ≥100 cpm for 60s epoch	4 days	WC, systolic and diastolic blood pressures ,HDL-cholesterol, C-reactive protein, triglycerides, plasma glucose and insulin	Yes	Total sedentary time associated with WC, HDL-cholesterol, C-reactive protein, triglycerides and insulin. Breaks were beneficially associated with WC, fasting plasma glucose and C-reactive protein.
Bankoski et al.(78) 2011 Netherlands Cross-sectional Study	665 with metabolic syndrome aged 71.0 (7.4). 702 without metabolic syndrome aged 71.0 (8.0)	Actigraph	A sedentary break was defined as an interruption in sedentary time ≥100 cpm	4 days	WC, triglyceride, HDL cholesterol, fasting glucose, systolic blood pressure and/or diastolic blood pressure.	Yes	A higher percentage of total sedentary time was associated with a significantly greater of metabolic syndrome. Fewer sedentary breaks were associated with a higher likelihood of metabolic syndrome. People with metabolic syndrome spent more hours as sedentary, had a longer average sedentary bout and fewer breaks in sedentary time.
Healy et al.(42) 2008a USA	168 adults aged 53.4 yrs (11.8)	Actigraph	Sedentary time broken if accelerometer counts were ≥100 cpm for 60s epoch.	5 days	WC, BMI, serum triglycerides, HDL cholesterol, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, and 2-h plasma glucose.	Yes	The breaks were beneficially associated with WC, BMI, triglycerides, and 2-h plasma glucose.

1.7 Physiological Mechanisms and Acute Effects of Sedentary Behaviour and Breaks in Sedentary Behaviour on Health

1.7.1 Human Studies

In 20 healthy adults (14 men, 6 women), Hamburg et al. (184) observed the effect of 5 days of complete bed rest on insulin sensitivity, total cholesterol, plasma triglycerides, glucose, and on vascular function. Insulin sensitivity was measured by a glucose tolerance test and vascular function by ultrasound and venous occlusion plethysmography. The baseline results were within normal limits in all participants. At the end of the study, there was a significant increase in the total cholesterol, plasma triglycerides, glucose, insulin resistance, increased blood pressure, and impaired microvascular function without a change in body weight. Likewise, Yanagibori et al. (185) observed a significant increase in plasma triglycerides, and a significant decrease in HDL cholesterol levels in 10 healthy adults (5 men and 5 women), without a change in their body weight, following 20 days of bed rest. Therefore, an extended period of sedentary behaviour may have adverse consequences on metabolic health (184). The findings from the above studies support a role of SB in increased cardio-metabolic risk. However, these situations of total bed rest for days are a level of extreme inactivity, and do not represent typical SB displayed by free-living humans (185).

Dunstan et al. (186) observed that interrupting 5 hrs sitting with 2 min bouts of light or moderate intensity every 20 min in 19 overweight/obese adults (11 men, 8 women; mean age, 53.8 yrs (4.9)) resulted in a reduction (light: 24%; moderate : 30%) in postprandial glucose, and a 23% reduction in insulin for both light activity and moderate activity compared with uninterrupted sitting for 5 hrs (186). Peddie et al (189) conducted a study on 70 healthy adults of the effects of prolonged versus shorter periods of sitting. They studied in 3 conditions in a random order, A: sitting for 9 hrs, B: sitting for most of this time but with interruptions of 30 min of walking, and C: breaking up the sitting time by walking for 1 min and 40s every 30 min. Peddie et al. (187) detected that both glucose and insulin were significantly lower in the participants who took regular

breaks compared to both the prolonged sitting and the 30 min continuous activity, while no differences were seen between the latter two. The triglyceride response did not differ between the three conditions (187).

Altenburg et al. (188) also observed similar findings in a study on 11 healthy adults (5 men, 6 women, age: 18-24 yrs) comparing two different conditions: A: sitting for 8 hrs; and B: sitting for 8 hrs interrupted with hourly, 8 min, moderate-intensity cycling exercise bouts. Altenburg et al. found that muscle activity (measured by electromyography) during cycling was seven to eight times higher compared with rest; postprandial levels of C-peptide were significantly lower during interrupted sitting compared with uninterrupted sitting. However, glucose, triglycerides, and cholesterol were not significantly different between both conditions (188).

However, Saunders et al. study (189) of 19 healthy children (11 boys and 8 girls) aged 10–14 yrs, looked at whether 8 hrs of uninterrupted sitting increases markers of cardio-metabolic disease risk (insulin, glucose, triglyceride, HDL and low density lipoproteins cholesterol) in comparison to 8 hrs of sitting interrupted with a 2 min light-intensity walk break or interrupted with structured physical activity every 20 min, with these 3 conditions performed in random order. Saunders et al. did not observe any acute effect of prolonged sitting, with or without interruptions, on markers of cardio-metabolic risk in healthy children and youth (189). It is not yet known whether these differing findings reflect differences related to age. To date, no study has examined the acute impact of prolonged sitting, with or without interruptions, on the health of young children.

While the above studies provide important insights, these studies cover only the short-term effects of prolonged sitting and breaking up prolonged sitting on health.

1.7.2 Animal Studies

As mentioned in Section 1.4, the findings on possible effects of sitting time and fragmentation in humans are supported by work in animal models, which suggested that prolonged sitting without interruptions resulted in significant reductions in lipoprotein lipase activity in skeletal muscle (36;104).

1.8 Measurement of Sedentary Behaviours

Accurate measurements of SB are necessary to assess relationships between SB and health outcomes, to gauge compliance with recommendations (e.g. in surveillance programmes), and to evaluate and determine the effectiveness of any intervention programmes (190;191). Moreover, accurate measurement is required to observe the changes in SB between and within individuals over time, e.g. in longitudinal studies, such as birth cohort studies. The development of accurate methods for measuring SB is the second of five stages of the SB epidemiology research framework (192). The five stages are: *1—establish links between behaviours and health; 2—develop measures of the behaviour; 3—identify influences on the behaviour; 4—evaluate interventions to change the behaviour; 5—translate research into practice* (192).

Any measurements used to assess SB in research programmes must be valid and reliable (190;191;193). In the following section, the methods of assessing SB in children are summarised in brief; subjective methods and objective methods will be outlined, and their validity and reliability considered.

1.8.1 Subjective Methods

Subjective methods use self-reporting instruments or, for young children particularly, proxy reports by parents to assess SB (59;190;194). In general, SB studies have mostly assessed TV viewing in adults, in children (38;190;194), and in young children (40). However, the measurement of TV viewing is not representative of all SB (e.g. using transport, sitting during leisure time or during work) (59;195;196), and is not the subject of this present thesis.

The questionnaires that have been used to assess sedentary behaviour, such as Previous-Day Recall of Active and Sedentary Behaviours, are valid tools to estimate the active and sedentary time of adults and adolescents (197;198). The Children and Adolescent Sedentary Activity Questionnaire provides information about the type, frequency and duration of the sedentary activity (199;200). The Sedentary Behaviour Questionnaire was designed to assess the amount of time spent doing 9 SB (watching television, playing computer/video games, sitting while listening to music, sitting and talking on the phone, doing paperwork or

office work, sitting and reading, playing a musical instrument, doing arts and crafts, sitting and driving/riding in a car, bus, or train) during weekday and weekend (201;202). The Children's Leisure Activity Study (in both self-complete and proxy parent formats) gives information about type of leisure physical activity, type of sedentary activity, frequency and total time spent in each activity during weekday (203).

As with subjective methods of measuring physical activity, subjective methods for sedentary behaviour have been used in many studies, and may give useful information about the type and context of the behaviour (watching television, playing video games, reading and using transport) of SB (40;54;80). They can be used in large populations because of the relatively low cost (190). However, these methods have limitations, described previously in section 1.2.1.3. Further validation studies are needed to demonstrate the use of the subjective methods for estimating the sedentary time (190;204) and combinations of subjective methods with objective methods such as accelerometers have been recommended for future SB studies (200;205).

1.8.2 Objective Methods

Because of the limitations of subjective methods, objective measurements are now being widely used in the current studies into SB. These methods are generally considered the best methods currently available for quantifying amounts of SB. The objective methods of assessing SB will be summarised in the section below.

1.8.2.1 Direct Observation

Direct observation can be used as a criterion measure (gold standard) for SB research (59;190). In direct observation, the intensities of posture or posture transitions are described visually.

There are different direct observational systems available, e.g. the children's activity scale (CARS) (where SB was defined as level 1 & 2 "sedentary & sedentary and movement of the limbs or trunk" (59;206)) and the children's

physical activity form (CPAF) where SB was defined as level 1 & 2 in a 4-point scale as "stationary with limb movement but no trunk movement" (207).

Direct observation can also include videoing the participants for later review. In some recent studies (208-211) using direct observation, the participants have been videotaped, with visual categorisation being carried out later. For example, Davies et al. (208) used direct observation (as a gold standard) to validate the activPAL™ for measurement of ST and breaks in ST in pre-school children. In this study, the children were videoed (direct observation) for one hour in a nursery while wearing the activPAL™; the authors concluded that the activPAL™ is a valid measure of ST in children (208). (This study is described in more detail in Section 1.8.2.2).

Direct observation provides rich information on the participants' SB and posture changes. However, it is a time-consuming method, and it is not suitable for large-scale studies (59;205).

1.8.2.2 Accelerometers

Accelerometers are sophisticated electronic devices that can measure both SB and physical activity objectively (46;54). As the subject of this thesis is mainly breaks in SB, the three monitors (Actigraph, Actical, and activPAL™) that have been widely used to measure SB and breaks in SB in research studies are described below:

The Actigraph

At present, the Actigraph is the most commonly used accelerometer for measuring SB objectively in all age groups (54;209). The Actigraph is a small uniaxial monitor (size: 38x37x18mm, weight: 27g). It is a non-waterproof device, and is usually worn on the hip by using an adjustable belt (209;212).

In earlier models, the Actigraph did not include an inclinometer for detecting postures, and the Actigraph could not therefore distinguish between sitting and standing. Some earlier studies using this device, therefore, counted standing as sedentary time (210;211). Newer Actigraph models (GT3X and GT3X+) have an inclinometer. However, misclassifications may still occur with standing

misclassified as sitting (190;211;213-215). This may arise because the ActiGraph output is similar for standing still and sedentary activities, leading to misclassification of standing time as sedentary time (211). Further work will be needed to define a suitable technique that uses both the intensity and the inclinometer output of the GT3X+ in combination to more clearly separate standing and sedentary behaviour (215).

The Actigraph records data in user-defined epochs. An epoch is a defined time period over which accelerometer counts are averaged (216). The effect of varying epoch on sedentary time measurement has been investigated in some studies with inconsistent results: some studies reported that the choice of epoch had no effect on the amount of sedentary time (54); other studies concluded that there was a significant effect of epoch on determining sedentary time, and it was necessary to specify epoch during the device initialisation. Using shorter epoch has been recommended, as data collected under shorter epochs can be summed into longer epochs, but not vice versa (190;217;218). Newer Actigraph models e.g. the GT3X (with sampling frequency range from 30 to 100 Hz) allow the epoch to be determined during post processing of the recorded raw data. This may help to compare data of studies which use different epochs (219). However, to date, no study using Actigraph has examined the influence of different epochs on the measurement of breaks in sedentary time in children and adults.

The ActiGraph has traditionally been used to measure SB by identifying time spent in low intensities of activity. Defining intensity of movement using accelerometers such as the Actigraph has generally involved applying cut points to accelerometer output, and there has been vigorous debate about the number of counts that indicate different levels of activity and SB in both children and adults (46;54). Different cut points for defining SB from the Actigraph are in the literature. For example, the 100 cpm cut point in 60s epochs, defined by Evenson, is widely used to define SB in adults (210;211) and children (209). However, Keadle et al. (99) recently investigated the ability of Actigraph monitors with both the commonly used cut point of 100 cpm in 60s epochs, and additional cut points of 50, 150, 200, and 250 cpm in 60s epochs, for measuring SB in 20 adults (mean age 46.5 yrs, SD 10.7). The participants were directly

observed for two 6-h periods while wearing the monitor. The Actigraph with a 100 cpm cut point underestimated sedentary time by 4.9% compared with direct observation; the 150 cpm cut point had the lowest percent bias of 1.8%. (The percentage bias was calculated as [(monitor sedentary minutes/direct observation sedentary minutes) - 1 x100]. Positive biases indicated overestimates of SB and negative values indicated underestimates of SB. The percentage biases for the 50, 200, and 250 cut points ranged from - 22% to 17.8% and were higher than the commonly used 100 cpm cut point (- 4.9%) (99).

De Decker et al. (209) tested the 100 cpm cut point in preschool children. Forty-five preschoolers (mean age 5.49, SD 0.59) were videotaped while wearing the ActiGraph for 1 hr during classroom activities at preschool. The results indicated that the ActiGraph with the 100 cpm cut point overestimated the mean time spent in sedentary activities by almost 10% in this age group (209).

However, Janssen et al (220) recently presented validation study data from 40 pre-school children (mean age 5.3, SD 1.0). Here, the children wore the ActiGraph, initialized to collect data in 15s epochs, and were videotaped while following a 150-min activity protocol within a whole body room calorimeter to measure the energy expenditure. ActiGraph cut-points for SB defined by Evenson (≤100 cpm), Pate (≤148 cpm), Puyau (≤799 cpm) , Reilly (≤1,099 cpm), Van Cauwenberghe (≤1,488 cpm) and Sirard (≤1,592 cpm) were all compared. The 100 cpm cut point for measurement of time spent sedentary showed significantly higher accuracy for classifying SB compared to all others (P = 0.05). Classification accuracy was estimated by calculated sensitivity, specificity, and area under the receiver operating curve (ROC-AUC). ROC-AUC values were defined as excellent (0.9–1.0), good (0.8–0.9), fair (0.7–0.8), or poor (< 0.7). The sensitivity (Se %), specificity (Sp %), and area under the receiver operating curve of the different cut points were as follows; Evenson (86.7, 72.9, 0.80). Pate (89.2, 67.3, 0.78). Puyau (97.3, 47.2, 0.72). Reilly (98.2, 39.2, 0.69). Van Cauwenberghe (98.2, 31.5, 0.65) and Sirard (98.3, 29.9,0.64), respectively (220). This suggested that the Evenson cut-points were a satisfactory choice for this age group. Similarly, Trost et al. (221) found that the ≤100 cpm is a good cut point for defining SB in 5–15 yrs olds, compared with other cut points. Moreover,

Trost et al. (222) suggested that the 100 cpm threshold for SB may be the most practical choice among toddlers and preschoolers.

Atkin et al. (223) have observed that the choice of cut point influences associations of objectively measured SB using the Actigraph with health outcomes in both children and adolescents. In general, there was a stronger association between sedentary time and metabolic risk observed when a higher Actigraph cut point was used to define sedentary behaviour, but the authors were of the view that the results of studies using different cut points should be compared with caution. It seems that further studies examining the associations between sedentary time and metabolic health across different accelerometer cut points are likely to be needed (223).

The activPAL™

The introduction of devices that also have inclinometers has transformed the ability to detect posture changes. For example, the activPAL™ monitor (PAL Technologies Ltd., Glasgow, UK) includes an inclinometer, and can provide information on time spent sitting, lying, standing, and walking, as well as recording the number of steps and cadence (steps/min) (68). The activPAL™ measures acceleration at a sampling frequency of 10Hz. The activPAL™ monitor is usually attached to the thigh. When the monitor is horizontal (with the participant lying or sitting), the acceleration is recorded as 0. Acceleration is recorded as equal to acceleration due to gravity when the monitor is vertical (participant is standing); when walking or moving, a greater acceleration is imposed, (Figure 1.3) (68). By default, a minimum seated duration of 10s is required to be classified as sitting/lying (manufacture's default settings, i.e. 10s MSUP). This activPAL™ setting can be changed manually from 1s to 100s MSUP (208). (Changing the MSUP in the activPAL™ software is in Section 2.2.2).

The activPAL™ has previously been validated for the measurement of ST in adults (210;224) and children (101;103;208;209) - studies that will be discussed in the section below.

Grant et al. (224) examined the validity of the activPAL™ against direct observation to measure ST in 10 adults (mean age 43 yrs, SD 10.6) in a

laboratory environment. The mean percentage difference between sitting time from the monitor and direct observation was 0.19% (224). Kozey-Keadle et al. (99) reported that the correlation between the direct observation and the activPAL™ percent of time sitting was high (R2 = 0.94) in a study of 20 adults (mean age 46.5, SD 10.7) who were videoed for two 6-h periods during working hours while wearing an activPAL™ (99). Hence, the activPAL™ appears to be a valid tool for measuring ST in adults (190;210;224). In both the above studies (210;224), a default setting of 10s MSUP was used.

In children, to date four studies (101;103;208;209), three in young children (103;208;209), have examined the validity of the activPAL™ for measuring ST against a criterion measure. Studies examining the reliability and criterion validity of the activPAL™ for measuring ST in adolescents do not currently exist.

Aminian et al. (101) examined the validity of the activPAL™ monitor using the default settings (MSUP 10s) against video observation (for 30 min) in measuring ST in 25 children (mean age 9.9 yrs, SD 0.3). A perfect correlation (r = 1.00) between activPAL™ data and video observation in time spent sitting/lying was noted (101).

In young children, initially, Davies et al. (208) validated the activPAL™ with a 1s MSUP against direct observation (one hour duration) to detect ST during usual nursery activity in 30 pre-school children (mean age 4.1, SD 0.5). The results were positive, but less good than in the adult studies; the sensitivity for sitting/lying was 99.5% with a positive predictive value of 99.5% in adults, and in the children's studies the sensitivity for sitting/lying was 87% with a positive predictive value of 96% (208).

In the study by Davies et al (208), sensitivity was calculated as [total number of seconds 'true positive']/[total number of seconds 'true positive' + 'false negative'] x 100. Positive predictive value was calculated as [total number of seconds 'true positive]/ [total number of seconds 'true positive' + 'false positive'] x 100. True positives were defined as all time-matched seconds in which the monitor output category and the direct observation category were identical. False positives were defined as all time-matched seconds in which the monitor output detected the category of interest but this did not agree with

direct observation. False negatives were defined as all time-matched seconds not detected by the monitor as the category of interest despite being in this category according to direct observation(208;225).

De Decker et al. (209) also have presented validity data from 44 preschool children (mean age 5.49, SD 0.59), videoed for 1 hr undertaking usual activities in nursery school while wearing an activPAL™ with a 10s MSUP. However, results showed low classification accuracy for the activPAL™ (area under the receiver operating characteristic curve, 0.6) in measuring ST; sensitivity was 53.8%, and the specificity was 67.5% (209). More recently, Janssen et al. (103) assessed the criterion validity of an activPAL™ with a 1s MSUP for defining sitting time against direct observation in 38 preschool children (mean age 5.3 yrs, SD 1.0), who followed a 150 min structured activity protocol in the laboratory. Findings indicated that the activPAL™ demonstrated good classification accuracy for sitting (area under the receiver operating characteristic curve, 0.88), sensitivity was 87.6%, and the specificity was 88.1%. These values are slightly lower than those found in the study by Davies et al. (208). The difference between these studies might be due to differences in how certain activities (other) were interpreted by the direct observation method (103). The lower classification accuracy for sit/lie in the study by De Decker et al. (209) might also be due to the use of a 10s MSUP, a setting that might not be suitable for young children. To date, the influence of the change of MSUP on sitting time components, and the most appropriate MSUP for the different age groups, is not known.
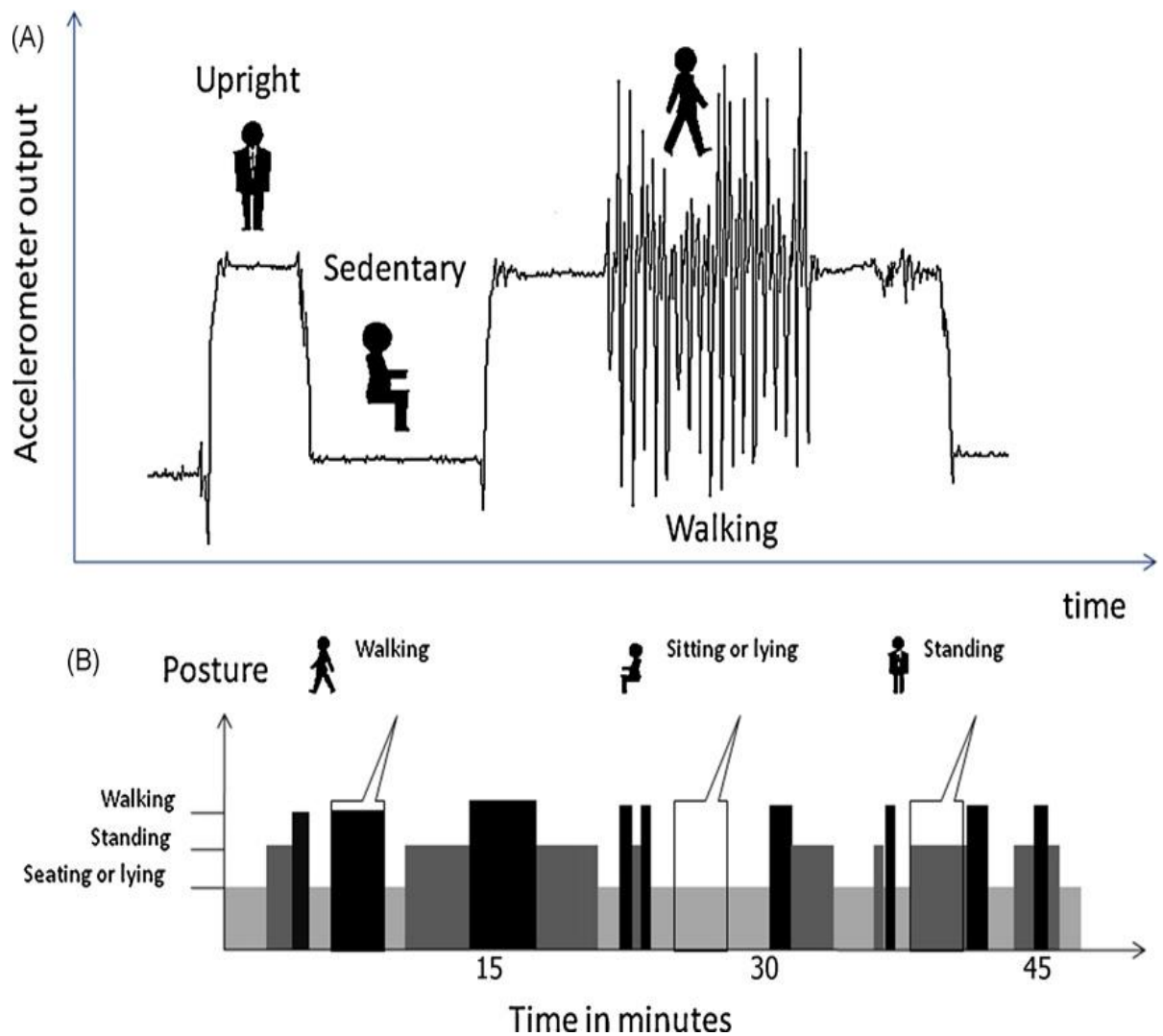
Figure 1.3 (A) Typical signal from a thigh mounted activPAL™ depending on posture. (B) Pattern of activity derived from the accelerometer signal by the propreitory activPAL™ software (A). Adopted from reference (96).

The Actical

The Actical is a small, omni-directional monitor hip-mounted accelerometer (28mm×27mm×10mm; 17 g). It is water resistant. Data can be saved in 15s epoch intervals (226). This monitor is able to measure movement in all directions - x, y, and z axis (although, it appears that it only measures one axis at a time). The Actical is used for objectively measuring the SB of adults (227) and children (226). However, evidence about the most appropriate cut point used to distinguish sedentary time is lacking, and it may misclassify standing time as sedentary time (226). In addition, the Actical is not able to provide raw data in any time less than a 15s epoch (228).

1.8.2.3 Heart Rate Monitoring and Indirect Calorimetry

Recently, heart rate has also been used to estimate SB in adults (229) and children (230). Sedentary time in most studies that used heart rate was measured as all heart rate observations below the Flex heart point, a discriminatory threshold between rest and exercise. Miss-classification of sedentary time can be reduced by changing the Flex heart rate (190;229). The combination of heart rate monitoring with accelerometry may improve the accuracy of activity measurement (231) and may permit discrimination of non-wear time from sleep/sedentary time (e.g. when the accelerometer records no counts but the heart rate record is obtained). However, heart rate is affected by environmental and emotional factors (229). Additional validation studies are required (190). At present, heart rate on its own is not used widely in the measurement of sedentary time in children (232), and it has particular problems with lack of compliance in pre-school children and so was not considered suitable for the studies in this thesis.

Indirect Calorimetry (measuring energy expenditure) has also been used as a criterion method in SB validation studies, to measure EE, oxygen consumption and carbon dioxide production were measured continuously in a room calorimeter while the participants were following the study activity protocol in the room (220;233). Resting EE was calculated by dividing measured EE for each participant by their individually estimated basal metabolic rate (BMR) using an equation. EE were classified based on their equivalent MET values of different

activity intensities: sedentary level was defined as ≤ 1.5 times predicted BMR (220). As mentioned before in section 1.2.1.1, this method is an accurate method but, it is limited to laboratory experiments.

1.9 Measurements of Breaks in Sedentary Behaviour

1.9.1 The Measurements of Breaks in Sedentary Behaviour in Adults

At the present time, researchers have used the ActiGraph and activPAL™ to measure breaks in sedentary time in adults.

1.9.1.1 The ActiGraph and the Actical

Healy et al. (42) first used the ActiGraph to measure breaks in SB to assess the relationship between breaks in SB and health, independent of the total amount of SB, in adults. They defined a break as an interruption in sedentary time (≥100 cpm), a cut point first suggested by Healy et al. (42) After Healy et al., some studies used the ActiGraph and the Actical, with the same definition of breaks in SB (78;81;83;84;119), to quantify the number and length of breaks in SB in overweight and obese women and hence to investigate the association between breaks and health outcomes (79). There have also been intervention trials targeting sedentary time reductions and an increase in breaks in sedentary time in older adults (82). The ActiGraph has even been used as a criterion measure to validate a new interviewer-administered questionnaire measure of workplace sedentary time and breaks in SB (80).

Lyden et al. (211) validated the activPAL™ and ActiGraph for estimating sedentary time and the number of breaks in sedentary time against direct observation (the criterion method) in 13 adults (mean age 24.8 yrs, SD 5.2). This was done for approximately 10 consecutive hours on two separate days in a free-living environment, using a programmed hand-held personal digital assistant (PDA) (211). Breaks in ActiGraph data were defined as any instance where a minute identified as sedentary (cpm <100) was followed by a minute identified as non-sedentary (cpm ≥100). For activPAL™ data; breaks were defined as a sit/lie that was followed by a stand or step. Lyden et al found that the

activPAL™ accurately estimated the absolute number of breaks in ST, but the ActiGraph significantly overestimated breaks in SB in the adult age group (211).

1.9.1.2 The activPAL™

The previous validation studies by Grant et al. (224) and Lyden et al. (211) indicated that the activPAL™ is a valid tool to estimate the number of transitions between sitting and standing (breaks in ST) in both laboratory (224) and free-living settings (211). Grant et al. (224) reported that the total number of breaks in ST were identical between the observation and the activPAL™ monitor, with an overall agreement of 95.9%. Lyden et al. (211) also found that the activPAL™ accurately estimated the absolute number of breaks in ST (bias, 0.3% (-7.0 to 7.7)) in free-living conditions. Thus, there is evidence that the activPAL™ is a valid device for measuring the number of breaks in ST in adults (211;224). As a result, researchers have started using the activPAL™ monitor to measure breaks in total sitting time, and to compare breaks in sitting between two groups, healthy individuals, and individuals with chronic health conditions. For example, one study found that there was no difference in total sitting time, but there were significant differences in breaks in ST occurring between three groups of adults: healthy participants with a sedentary occupation, participants with chronic low back pain, and participants with chronic fatigue syndrome (96).

The activPAL™ has also been used in the measurement of ST in intervention studies (pre- and post- intervention) as a study outcome (97-99). In one study, the aim was to explore an intervention by computer software reminders to stand up while at work were used to reduce long uninterrupted sitting periods (234). In a second study, participants received information targeting reducing sedentary time and where and when this could happen (98;99). Both studies involved adults. The results of these studies suggested that such approaches may encourage adults to reduce their sitting behaviour (97-99). They also observed that the activPAL™ was sensitive to the reductions in ST and therefore may be a suitable outcome measurement tool to be used in such intervention studies (235).

Harrington et al. (100) observed total sitting time, number and length of sitting bouts, and breaks in sitting activity among adolescent girls using the activPAL™

and found that longer bouts of sitting time were accumulated during school compared to after school. There was however, no difference in total time spent sitting. This study was also discussed in section 3.6.2 (100).

1.9.2 The Measurements of Breaks in Sedentary Behaviour in Children

So far, only a few studies have objectively measured breaks in sedentary or sitting time in children and youths, using mainly the Actigraph, Actical, or activPAL™. The available studies are summarised in Tables 1.3, and 1.4, according to which monitor had been used in the study, and these are summarised briefly below:

1.9.2.1 The Actigraph

Five cross-sectional studies and two longitudinal studies (Table 1.4) used the Actigraph to measure breaks in sedentary time in children. In these studies, breaks had been defined as suggested by Healy et al. in the adults' study, where sedentary time is broken if accelerometer counts were ≥100 cpm for a 60s epoch (42). Studies in Table 1.4 examined the associations between accelerometer-measured breaks in SB and health risks in children and youths (86;90;92-95), and described changes in the frequency of sedentary breaks during childhood and adolescence (88) . However, no study has validated the ability of the Actigraph to measure the breaks in sedentary time in children and young people.

1.9.2.2 The Actical

The remainder of the studies in Table 1.4, (three cross-sectional studies) (85;89;91), used the Actical to observe the association between breaks in SB with any health markers, such as BMI, WC, blood pressure, and HDL cholesterol (89;91). They also used the Actical to describe the patterning of SB (bout length and breaks in sedentary time) of children at and away from school (80).

While it seems that no study has investigated the ability of the ActiGraph or Actical to measure breaks in sedentary time in children and youths, researchers have started using these monitors to assess the associations between breaks in SB and health outcomes (Table 1. 3, Table 1.4). Moreover, a recent study of

adults by Lyden et al. (211), the study mentioned in Section 1.9.1.1, concluded that the ActiGraph is not a valid tool to measure breaks in sedentary behaviour.

Therefore, while the Actigraph and Actical have been used often to measure breaks in sedentary time in children, their validity is unclear.

1.9.2.3 The activPAL™

Aminian et al. (101), researching into children aged 9-10 yrs, validated the activPAL™ against direct observation in measuring breaks in ST in a laboratory setting (Table 1.3). The authors found a high correlation (r = 0.99, 90% Confidence Limit) between the activPAL™ monitor data (the activPAL™ file summarises data into 15s epochs was used), and the video observation of the total count of sit to stand and stand to sit transitions. The authors concluded that the activPAL™ is a valid tool for measuring breaks in ST in children at this age (101).

In contrast with the results from the above study, the two validation studies (102;103) of activPAL™ in young children against direct observation (criterion method), Table 1.1, observed that activPAL™ overestimated the number of breaks in ST with a 1s MSUP. Davies et al. (102) found that the activPAL™ overestimated the number of directly observed breaks (direct observation mean = 6; activPAL™ mean = 9; p <0.01), There was a significant rank–order correlation (r = 0.79, p <0.0001) between the number of breaks measured by activPAL™ and by direct observation in free-living conditions (102).

Similarly, Janssen et al. (103) found the total number of breaks in ST was significantly overestimated (p <0.01). The above two studies used a 1s MSUP to better capture the rapid transitions that may be made by young children (103). The difference between the studies in young children and the study in older children may be due to the differences in breaks in different age groups. Additionally, the 1s MSUP, which has been used in young children's studies (102;103) , may not be suitable for an older age group.

In light of Lyden et al.'s study (211) suggesting that the ActiGraph is not a valid tool, more evidence is needed to examine the validity of available monitors to

assess breaks in sedentary or sitting time in different age groups. The lack of valid monitors is a significant barrier, since there is a clear research need to clarify the association between breaks and health outcomes, to record the patterns and changes in breaks in SB between and within individuals over time, and also to establish recommendations and plan interventions.

Table 1.3 Validation studies and observational study using the activPAL™ to measure breaks in sitting time in pre-school children

Study	Study group	Monitor	Breaks	Duration	Conclusions
Janssen et al. (103) 2013, Australia Validation Study	38 pre-school children (mean age 5.1 yrs, SD 1.1) (range 4-6)	The activPAL™	Sit/lie to upright (stand or walk) transitions, using a custom-made Microsoft Excel, version 2010 macro using the second-by-second activPAL™ posture allocation data, with a 1s MSUP	Children were filmed (criterion method) during the study protocol for 150 min, Most activities lasted 3 to 5 min	Time spent sitting and standing was overestimated by the activPAL™ while time spent walking was underestimated. Total number of breaks in sitting time was overestimated significantly p <0.01
Davies et al. 2012, UK (102) Validation Study	30 pre-school children (mean age 4.1 yrs) (range 3.1–4.9)	The activPAL™	Sit/lie to upright (stand or walk) transitions, using HSC PAL analysis software (version 2.14) with a 1s MSUP	1 hr was videoed with filming (criterion method) of usual activity at nursery	Total number of transitions in sitting time was overestimated significantly p <0.01

Table 1.3 (Continued) Validation studies and observational study **using the activPAL™** to measure breaks in sitting time in children

Study	Study group	Monitor	Breaks	Duration	Conclusion
Aminian et al. (101) 2012, New Zealand Validation Study	25 children 9-10 yrs (mean age 9.9, SD 0.3)	The activPAL™	The total number of sit-to-stand and stand-to-sit transitions using the activPAL™ file summarizes data in 15s epochs	Children were filmed (criterion method) during the study protocol for 30 min (5 min sitting, 5 min standing, and 5 min walking). In addition, a 20-step walking test was performed. This process was repeated twice.	The activPAL™ monitor is a valid tool for measuring time spent sitting/lying, standing, and walking, and the total count of sit-to-stand and stand-to-sit transitions along with step counts.
Hinckson et al. (87) 2013, New Zealand. Cross-sectional	56 children (mean age 10.2, SD 0.9) (9–10 yrs of age)	The activPAL™	The total number of sit-to-stand transitions, using the activPAL™ file summarizes data in 15s epochs.	For 14 days	Children's sitting/lying, standing and walking time changes from week-to-week by a small amount. Step-count data followed a similar trend, but the sit-to-stand count changes were either unclear or trivial.

Table 1.4 Observational studies **using the Actigraph** to measure breaks in sedentary time in children and youths

Study	Study group	Monitor	Breaks	Duration	Health outcome	Adjusted PA	Conclusion
Altenburget al.(92) 2014 Netherlands Cross-sectional Study ENERGY (EuropeaN Energy balance Research to prevent excessive weight Gain among Youth)	647 European aged 11.6 (0.8) yrs	Actigraph	accelerometer counts were ≥100 cpm for 5s epoch	6 days	WC, glucose, C-peptide, LDL-cholesterol, HDL-cholesterol and triglycerides	Yes	**No association** between total sedentary time or sedentary time accumulated in bouts and cardio-metabolic health
Carson et al. (93) 2014 Canada Cross-sectional Study	787 children aged 11.0 yrs	Actigraph	Sedentary time broken if accelerometer counts were ≥100 cpm for 60s epoch	4 days	BMI	Yes	**No association** between volume of SB, number of sedentary breaks and BMI. Minutes spent in sedentary bouts lasting 5–9 min during weekdays was positively associated with BMI, while minute spent in sedentary bouts lasting 1–4 and 5–9 min during weekend was positively associated with BMI
Saunders et al. (90) 2013, Canada Cross-sectional Study	522 children aged 9.2 (range 8.0–11.0 yrs)	Actigraph	Breaks in sedentary time were calculated as any interruption in sedentary time lasting 60s epoch or longer in which the accelerometer cpm ≥100	7 days	WC, BMI Z-score, fasting insulin, fasting glucose, triglycerides, HDL-cholesterol and C-reactive protein	Yes	**No association** between volume of SB and markers of cardio-metabolic risk. Breaks in sedentary time and short bouts of SB are independently and beneficially **associated** with markers of cardio-metabolic risk in children with a family history of obesity

Table 1.4 (Continued) Observational studies **using the Actigraph** to measure break in sedentary time in children and youths

Study	Study group	Monitor	Breaks	Duration	Health outcome	Adjusted PA	Conclusion
Kwon et al.(95) 2013, USA Longitudinal Study	554 participants at 8.0, 11.0, 13.0, and 15.0 yrs of age	ActiGraph	Sedentary time was considered to be interrupted or broken if accelerometry counts were ≥100 cpm for 60s epoch	4 days	Body fat mass	Yes	**No association** between the sedentary time ,frequency of breaks in sedentary time and adiposity
Chinapaw et al. (94) 2012, Dutch and Hungarian Cross-sectional Study	73 children, at age 10.0–12.0 yrs	Actigraph	Sedentary time broken if accelerometer counts were ≥100 cpm for 15s epoch	4 days	BMI, WC, fasting plasma glucose, C peptide, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides	Yes	**No association** between volume of SB, **sedentary bouts** and metabolic indicators
Kwon et al. (88) 2011, USA Longitudinal Study	613 Children and adolescence from age 5.0 to age 15.0 yrs	Actigraph	Sedentary time broken if accelerometer counts were ≥100 cpm for 60s epoch.	4 days	-	-	Breaks in sedentary time notably decreased during childhood and adolescence.
Carson et al. (105) 2011, Canada Cross-sectional Study	2,527 children and adolescents, aged 13.0 yrs (10.0-16.0)	Actigraph	Breaks, sequences of any duration with an accelerometer count ≥100 cpm for 60s epoch.	7 days	WC, BMI, systolic blood pressure, (non-HDL cholesterol), and C-reactive protein)	Yes	**No association** was observed between volume of SB, the number of bouts, the breaks in SB and cardio-metabolic risk factors

Table 1.5 Observational studies using the Actical to measure the breaks in sedentary time

Study	Study group	Monitor	Breaks	Duration	Health outcome	Adjusted PA	Conclusion
Abbott et al. (236) 2012, Australia Cross-sectional Study	53 children aged 10.0-12.0 yrs, (mean age 11.2 yrs, SD 0.8)	Actical	Breaks, sequences of any duration with an accelerometer count ≥100 cpm for 60s epoch	6 days	-	-	More than 2 hrs of each day was spent in sustained sequences of sedentary time, where their accelerometers did not register any significant hip movement for 30 min or more. School time in particular was associated with more uninterrupted sedentary time and fewer breaks than non-school time; however, the total school-day pattern was similar to weekend days.
Oliver et al. (89) 2013, New Zealand Cross-sectional Study	126 children aged 5.9 yrs (range 5.8–6.7yrs)	Actical	Breaks in sedentary time were defined as instances where the accelerometer counted ≥100 cpm for 60s epoch	3 days	WC	No	**No association** between volume of SB, breaks in sedentary time and WC
Colley et al.(91) 2013, Canada Cross-sectional Study	1,608 children and youths between the ages of 6.0 and 19.0 yrs	Actical	Breaks in sedentary time were considered as instances where the accelerometer counted ≥100 cpm for 60s epoch.	4 days	BMI, WC, blood pressure and non-HDL cholesterol	Yes	**No association** between volume of SB and cardio-metabolic risk factors. Prolonged bouts of sedentary time (less breaks) after the school period were **positively associated** with BMI and WC in boys aged 11 to 14 yrs. But no association was observed with any health markers in older or younger boys or in girls of any age

1.10 Thesis Aims

Differences in sitting time vs. standing time have been observed between obese and non-obese adults (237). Recent studies in adults indicate that, in addition to total SB, frequent breaks in SB may be beneficially associated with an individual's health in a number of dimensions. To date, studies investigating these findings are limited or do not exist in children. From the literature review presented above, the three monitors, the Actigraph, Actical, and activPAL™ have mainly been used to measure SB or ST and breaks in SB. However, validation studies only assessed the Actigraph and the activPAL™ for measure breaks in SB; the Actigraph has not been shown to be a valid tool for the measurement of breaks in SB in adults and, to date, no validation study has examined the ability of the Actigraph to measure breaks in SB in children.

One issue that emerges as needs clarification is a technical one vis the influence of minimum time of sitting necessary to classify a time interval as sedentary. Based on a small number of studies (101;224), the activPAL™, using a 10s MSUP, appears to be a valid tool for measuring the breaks in ST in older children, 9-10 yrs old, and adults (101;224). But it is not yet clear that it is a valid tool for young children, as the two studies (102;103) of young children had used the activPAL™ with a 1s MSUP setting to examine the ability of the activPAL™ monitor to measure breaks in ST. A 1s MSUP setting may not be suitable for young children, as, to date, the effect of the setting on the total ST and breaks in ST, and the most appropriate MSUP for different age groups, have not been systematically investigated. It, therefore, appears that there are a number of methodological issues that need answering before the activPAL™ can be used more widely. A particular issue is that the appropriate MSUP to define best breaks in sitting (transitions) for different age groups is not known. This is of some importance because the number of breaks in sitting time measured when using the activPAL™ might depend on the choice of minimum duration of any posture transition (103). As a primary barrier to elucidating the association between SB components, such as breaks in SB and health outcomes, the need for validated monitoring tools is important issue that needs addressing.

Therefore, in this thesis, the aim of Study 1 in Chapter 3 was to investigate the effect of systematic variation of the minimum setting time in the activPAL™ on both the total time spent sitting, and breaks in sitting. In Study 2 (Chapter 4), the aim was to determine the optimum activPAL™ setting of a MSUP for measuring the sitting time and breaks in sitting in young children.

It is important to study children's behaviour at a young age. This is based on the belief that behaviours and habits may be begun and established early in life as sedentary behaviours and physical activity have shown a tendency to track consistently over time from the preschool years to childhood (11;238-240) and sedentary behaviour appears to track more consistently than physical activity (11).Therefore the preschool period might provide a window of opportunity for interventions particularly in the light of widespread preschool education in many countries which may provide an opportunity to access the majority of this age group (172). Interventions targeting reduced time spent being sedentary during this period are being considered, and may be beneficial for future health outcomes (241;242).

The specific aims of the four studies performed in this thesis with the associated hypotheses are outlined as follows:

1.10.1   Study 1 - Chapter 3

Hypothesis: The change of the activPAL™ MSUP has an effect on the accuracy of measurement of the components of sitting (sedentary) behaviour, i.e. the total time spent sitting, breaks in sitting, and sitting bouts.

Aim: To examine the effect of variations in the activPAL™ setting on the components of sitting behaviour in young children using the measures recommended in adults.

1.10.2   Study 2 - Chapter 4

Hypothesis: The appropriate setting of MSUP to measure accurately the total time spent sitting, and breaks in sitting, is different for different age groups.

Aim: To determine the most appropriate MSUP for young children, using direct observation as the criterion method.

1.10.3 Study 3 - Chapter 5

Hypothesis: The differences in posture allocation observed between overweight/obese and non-obese adults may also be present in children.

Aim: To conduct a pilot study to investigate the differences in sitting behaviour components between overweight/obese and healthy weight children.

1.10.4 Study 4 - Chapter 6

Hypothesis: Direct measurements of both sitting and breaks in sitting will be different between boys and girls.

Aim: To conduct a pilot study to investigate whether sitting time and breaks in sitting differs between boys and girls.

Chapter 2

Generic Methods

2.1 Anthropometric Measures

The date of birth and sex were recorded for each child. The decimal age was calculated for each participant from their date of birth to the date of the start of data recording. Their height was measured to the nearest 0.1 cm with a calibrated stadiometer (Leicester Height Measure™). Children were measured standing up straight, with their heels and buttocks positioned in contact with the vertical backboard. Their head was in the horizontal plane position, arms at their sides, shoulders relaxed and legs straight with feet together on the centre base plate. The headboard of the apparatus was placed carefully on the child, and the measurement to the last complete millimetre was read from the instrument. To ensure accurate height measurements were taken, height was measured 2-3 times for each child and the mean was recorded.

Weight was measured to the nearest 0.1 kg by using a Seca™ scale. The children wore light indoor clothing and were asked to take off all their outer clothing and shoes. Height and weight measures were used to calculate body mass index using the standard formula, Weight (kg)/height^2 (m^2) (243). All the measurements (height, weight, and BMI) were then expressed relative to UK 1990 population reference data as a standard deviation score (SDS) for each child (243;244).

2.2 Measurement of sitting variables - sitting time, breaks in sitting, sitting bouts - using the activPAL™

The activPAL™ (PAL Technologies Ltd., Glasgow, UK) is a small (5.3cm x 3.5cm x 0.7cm) and lightweight (15g) accelerometer and inclinometer (Figure 2.1). It is a uni-axial monitor that measures acceleration in one direction, vertically through the monitor (68).
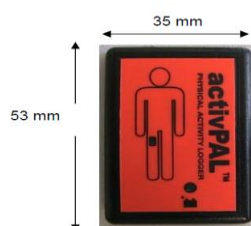
Figure 2.1 The activPAL™ monitor.

2.2.1 Charging and Programming the activPAL™

The activPAL™ is able to record data for 8 consecutive days, powered by an internal rechargeable battery. The device is recharged using a docking station. To charge the device, a docking cable from the docking station (Figure 2.2) is connected to a USB port on a PC (Figure 2.2a). Then the activPAL™ device is placed in the docking station in a charging slot [there are five stations on the docking cable, four for charging] (Figure 2.2b) and one for programming, data transfer, and also charging (Figure 2.2c). Charging takes around 3hrs for a full charge. An orange light on the device shows when the battery is charging, and switches off to indicate when the device is fully charged.

Prior to use on a participant, a fully charged activPAL™ is programmed to start recording new data. Programming the device requires the use of the activPAL™ professional software. This can be downloaded from the activPAL™ company website and installed on a personal computer. Once the software is installed, the device can be connected to the computer via a USB port using the station docking cable for programming (Figure 2.2c).

For programming, a device is placed in the programming site of the docking station connected to the computer with the software. The researcher starts the activPAL™ professional software, selects "Communicate with activPAL™" from the File menu, and presses the "reprogram and clear memory" button. After the memory is cleared, the reprogrammed device starts recording immediately. A series of rapid flashes indicates that the device is programmed and ready for use. If recording is to start at a later time, then the planned date and time of starting can be programmed using the software. Throughout the duration of the device recording on a participant, a green light flashes every six seconds to indicate that the device is active and recording (68).
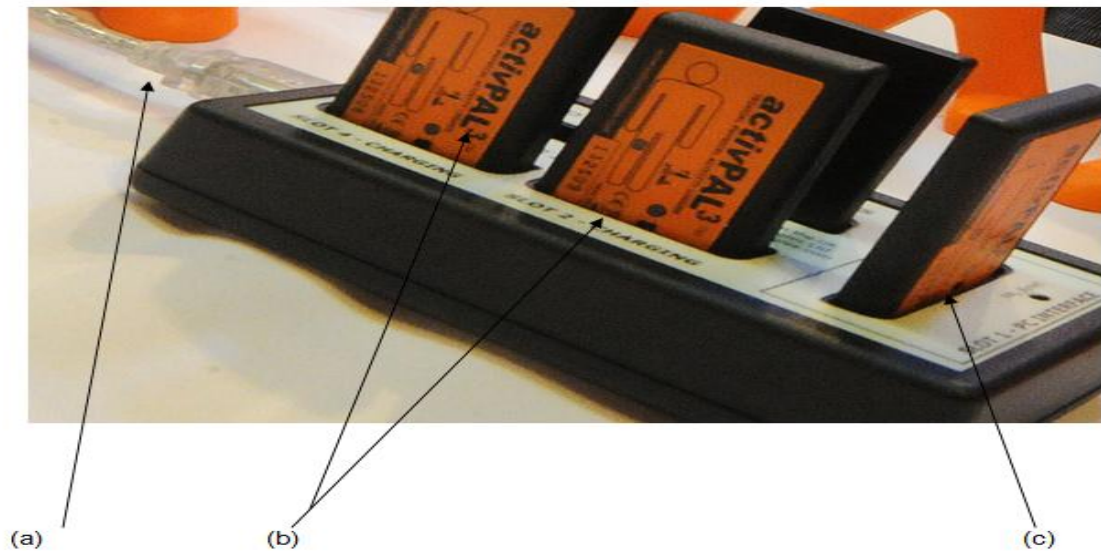
Figure 2.2 The docking station for activPAL™ devices: (a) the USB cable attached to the docking station; (b) devices being charged and (c) a device in the programming site.

2.2.2 Changing the Minimum Sitting/Upright Period (MSUP) in the activPAL™ software

As noted in the introduction above, although the activPAL™ is an event based system, the analysis software only counts breaks in sitting lasting longer than a user defined MSUP in the new posture.

This is intended to exclude very short postural "events" that are recorded by the monitor but are unlikely to have physiological meaning. Thus this setting effectively functions as a low-pass (high-cut) filter; (by default, this is set at 10s). Changing the MSUP in the activPAL™ software involves manually changing the setting in the range from 1s to 100s. This is done in the software by selecting tools from the file menu of the activPAL™, then selecting settings. A change only affects the time that the monitor waits to decide whether a posture is seated (Minimum sitting period) or an upright posture (Minimum upright period) (Figure 2.3). Changing the MSUP has no effect on stepping time. Steps are detected directly using a different algorithm that does not take the MSUP into account. Step measurement was not used in the present studies.
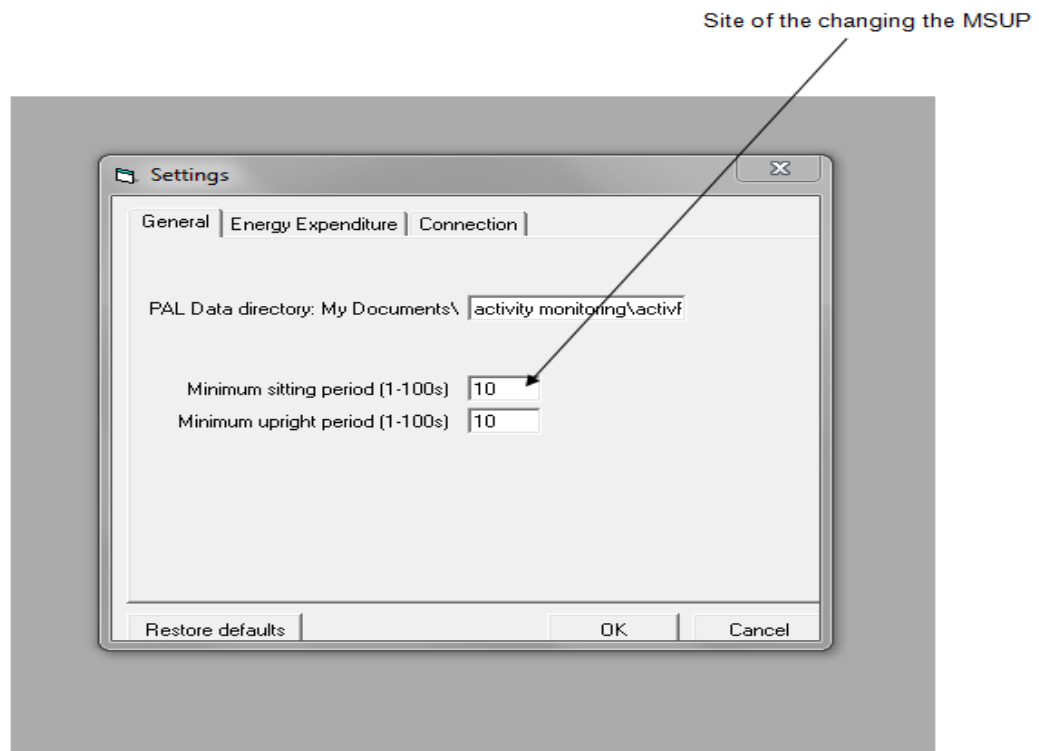
Figure 2.3 The window for changing the MSUP in the activPAL™ software.

2.2.3 The Placement of the activPAL™

Once charged and programmed, the device has to be attached to the child. The activPAL™ is placed directly onto a child's skin on their mid-thigh (Figure 2.4). The researcher then secures the monitor in place with a small hypoallergenic adhesive gel patch (hydro gel) "PAL stickie" (Figure 2.5). The device is further secured by applying a transparent sticky film (Tegaderm™) over the top (Figure 2.6). The activPAL™ monitor is then worn continuously for the required duration of monitoring. During monitoring, the child can continue wearing the device while carrying on with normal free-living activities during the day and during periods of sleep overnight.

Figure 2.4 The location of the monitor. The monitor is placed on the anterior mid-thigh.

Figure 2.5 PALstickie. Hypo-allergic adhesive gel patches (hydro gel).

Figure 2.6 Tegaderm. The transparent sticky film.

Present versions of the activPAL™ monitor are not waterproof (A sleeve to make the activPAL™ waterproof is now available, but when these studies started, these sleeves were not available). Accordingly, the monitor needs to be removed during any exposure to water. All parents were given written and verbal instruction about the fact that the monitor had to be removed temporarily during periods of showering, bathing, or swimming at the time the monitor was first sited by the researcher. Parents were provided with an activity recording sheet at the time the monitor was issued, and this was collected when monitoring ended. They were asked to record the date and time of both removal and reattachment any time the monitor was temporarily taken off and the reason for removal. The activity sheet did not ask parents to record sleep/wake times - an example of ActivPAL™ activity log is included as Appendix D. Children of this age will usually be sleeping in their own beds in a different room from their parents. This means that precise timing of sleeping or waking would not be routinely be known by the parents.

2.2.4 Duration of monitoring

Previous accelerometry studies have established that, for children, a minimum duration of device wear time to achieve reliable results is three weekdays for measurement of physical activity and sedentary behaviour , with at least 6 hrs of monitoring during waking hours per day (245;246). For the activPAL™, one study suggested that the 3 day reliability coefficient was 0.53 and for 4 day reliability was 0.87 (208). In Study 1b, Study 3, and Study 4, each child was asked to wear the activPAL™ monitor continuously, 24hrs a day, for between 5 and 7 days. In practice, in the present studies, the device wear time was always much greater than the minimum previously established for reliable data.

2.2.5 Stopping early if a child was unhappy

In normal circumstances, the monitor is small and very light and does not interfere with normal activity or play. However, occasionally a child was uncomfortable or unhappy wearing the monitor. If this occurred, and the parents could not persuade the child to continue wearing the monitor, the parents were advised to take off the monitor and end data collection.

2.2.6 Monitor collection and data download

At the end of monitoring period, the monitor was removed from the child and the device was returned to the researcher. The device was then inserted into the docking station (Figure 2.2c) and connected via the USB cable (Figure 2.2a) to a PC on which the activPAL™ Professional software was installed.

From the File menu, the researcher selected "Communicate with activPAL", and, once a connection had been established, pressed the "download store data" button to save the recorded data onto the computer. The data download usually took a few seconds to complete. The saved raw activPAL™ file for each child was identified by an anonymous code for further analysis.

2.3 Data reduction and operationalization of sitting variables,

The activPAL™ output classifies an individual's activity into three categories: "sitting/lying"; "standing" (standing with no movement); and "walking (steps)" (movement from one place to another). In addition, the activPAL™ identifies and counts posture transitions [sit-to-stand (u) and stand-to-sit (d)] (Figure 2.8). Data output can be presented per second, hour, day, week, or in 15 second epochs. In these studies, we made no use of the "15s epoch file" available with activPAL™ software.

The flow diagram below shows the data reduction procedure, figure 2.7
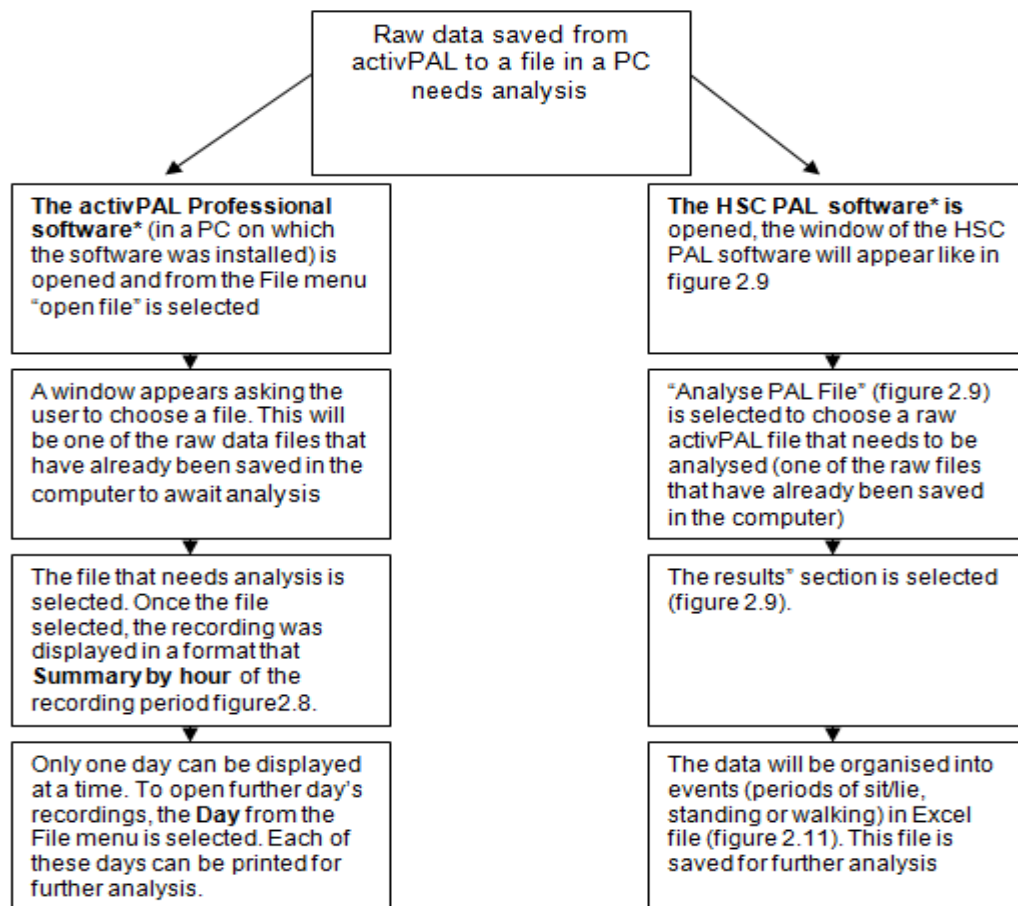
Figure 2.7 Flow diagram of the data reduction procedure. Total number of transitions sit-to-stand (u) was calculated from activity profile summarised by hour using activPAL™ Professional software. Total sitting time, number and duration of sitting bouts were calculated using activPAL™ HSCPAL analysis software. The missing time were deleted manually before any further analysis.

2.3.1 Missing data points

Non-wear time was identified from the parent/guardian activity recording sheet. The non-wear time is commonly represented by unchanging sitting/lying periods in the activPAL™ file (as the monitor was placed in a horizontal position while removed). Once the non-wear time was identified, the sum of the missing data was calculated for each child and then excluded from the recording before any data analysis was made.

2.3.2 Exclusion of weekend days

In Study 1b, Study 3, and Study 4, only week days were considered, and weekend days were excluded. This was done to avoid any effects arising out of different patterns of activity during weekend days (245;247).

It clearly would be interesting to explore the difference in the activity between weekdays and weekend especially as in a previous study by Page et al. (248) it was observed that the major differences in activity between obese/non-obese children were seen after school and at weekends (248).

Unfortunately, the populations of children studied were not homogeneous with respect to weekend activities. Some participants in this thesis were recruited from the Libyan schools (see section 5.3.1). Libyan schools take place during the weekends (from 10am until 4pm) and teach the Libyan curriculum. This curriculum has no PE activity, so children spend the majority of their time sitting during the weekend. This difference would clearly have biased the results if we had included weekend data.
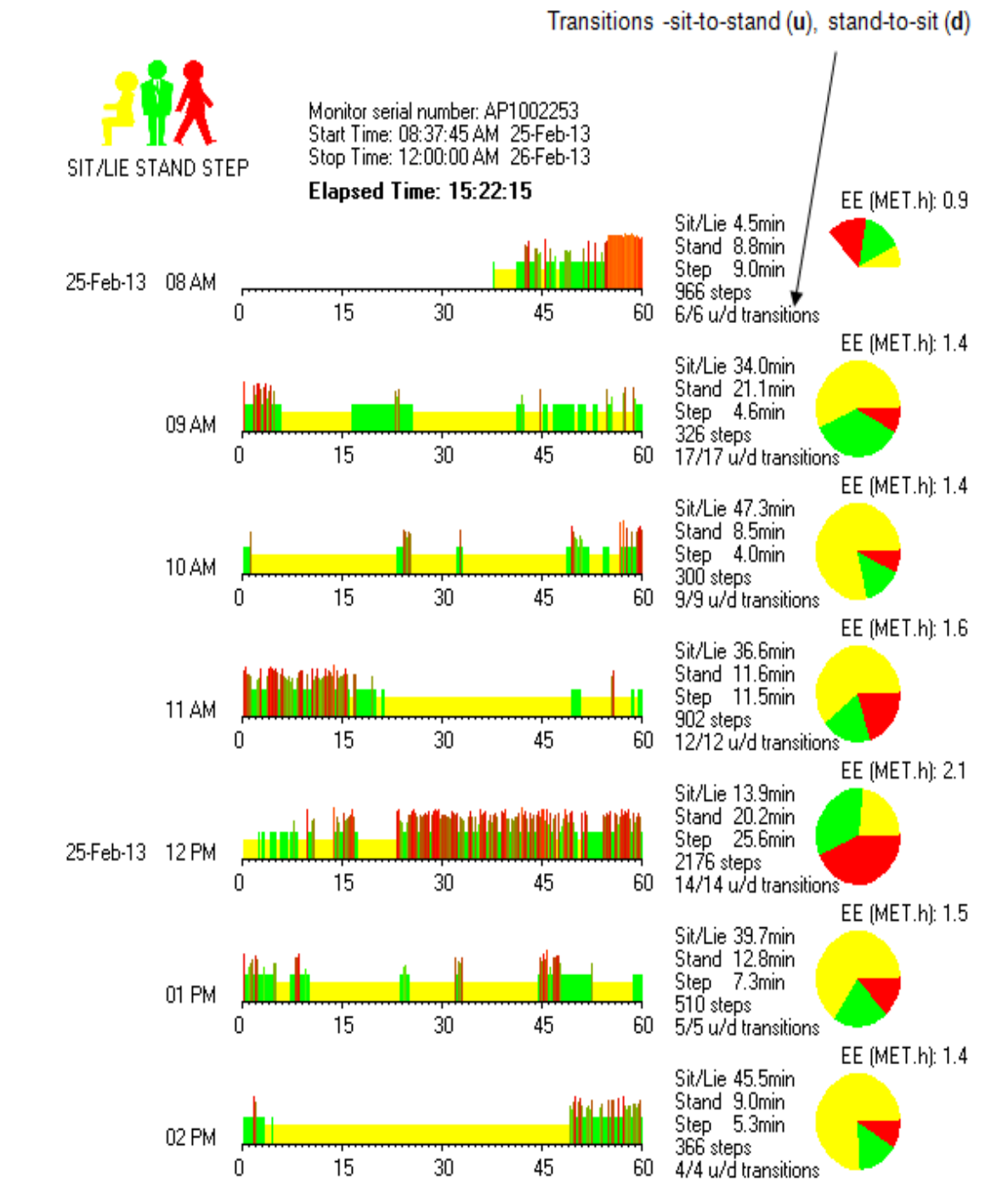
Figure 2.8 Example output of the activPAL™ (summarized by hour). Sit/lie periods are coded in yellow, standing periods in green, and walk-steps in red. Transitions [sit-to-stand (u), stand-to-sit (d)] are tabulated at the side.

2.4 Characterising sitting time

In these studies, sitting (sit/lie) was characterised in the following ways:

2.4.1 Total time sitting

Total time recorded as "sit/lie" during waking hours was calculated, using a custom software (HSC PAL analysis software) (Figure 2.9) (208), from the time of waking in the morning until the child went to sleep at the end of the day. This software was developed by Dall and Granat at Glasgow Caledonian University (HSC PAL software v 2.14) and allows detailed analysis of the activPAL™ output as classified by the original activPAL™ Professional Research Edition software (208). The HSC PAL software was provided by Dr Gwyneth Davies to the researcher. The HSC PAL software generates an event file (Figure 2.11) listing the time (in seconds) at which a change in output category (i.e. a transition) occurs. This is done by opening the HSC PAL software. The window of the HSC PAL software will then appear like in figure 2.9. The user clicks on "Analyse PAL File" to choose a raw activPAL file on which to run an analysis (one of the raw files that have already been saved in the computer, figure 2.10). The analysis takes a few seconds. The user then clicks on the results section (figure 2.9). The data will be organised into events (periods of sit/lie, standing or walking) in an Excel file (figure 2.11) which can be saved for further analysis for each child. Total sitting time during waking was calculated from this file. The data were sorted by colour for each day (figure 2.12) and the sum of sit/lie was calculated. Before any analysis was done the weekends were excluded and any missing time was deleted.

Waking time was defined from the first sit to stand transition in the morning, marking the fact that the child had risen from sleep; the researcher identified this transition by manual inspection of the event file (Figure 2.11). By manual inspection of the event files, the researcher also noted that the majority of the children studied were not active after 9pm. In light of this observation, the end of waking time was standardised at 9pm for all participants on all days of measurement. The total sitting time recorded as "sit/lie" during waking hours was then calculated as the time from waking time until 2100.

Site of introduce original pal file

| Analyse PAL file | Select Part of File | Sort Events |

File Statistics										
Full File Name										
File location										
	Whole file	1	2	3	4	5	6	7	8	9
Start Time	27/11/2012 13:11									
Stop Time	04/12/2012 13:12									
No. Events	11,563									
Total Time (s)	604,848									
Total time (days)	7.00									
No. Sedentary Events	2,161									
No. Upright Events	2,161									
No. Standing Events	5,781									
No. Walking Events	3,621									
Total Sedentary Time (s)	467,395									
Total Upright Time (s)	137,475									
Total Standing Time (s)	79,801									
Total Walking Time (s)	57,674									
No. of Transitions	4,321									
No. of Steps	70,424									
Average Cadence (steps/min)	73									
Total Energy (METs)	241									
	Prop Start									
	Event									
	Event Length (s)									
	Prop Stop									
	Event									

► ► Instructions Start Results Events Sorted Events Lists

Results

Figure 2.9 The window of the HSC PAL software – this allows the researcher to choose the file to be analysed.

ActivPAL raw file

Name		Date modified	Type
⭐ Favorites			
🖥️ Desktop			
🕘 Recent Places	AP060741 02Oct08 10-51-36 AM - 02Oct0...	05/06/2013 14:13	PAL File
📥 Downloads			
📚 Libraries			
📄 Documents			
🎵 Music			
🖼️ Pictures			
📹 Videos			
🌐 Homegroup			

File name: AP060741 02Oct08 10-51-36 AM - 02Oct08 12 ▾   pal files ▾

Tools ▾   Open ▾   Cancel

1	Total Upright Time (s)	137,475
2	Total Standing Time (s)	79,801
3	Total Walking Time (s)	57,674
4	No. of Transitions	4,321
5	No. of Steps	70,424
6	Average Cadence (steps/min)	73
7	Total Energy (METs)	241
8		Prop Start
9		Event
0		Event Length (s)
1		Prop Stop
2		Event

◄ ► ►| Instructions | **Start** | Results | Events | Sorted Events | Lists | ✏️

Figure 2.10 The window to select one of the raw files that has been already saved in the computer for analysis.

Figure 2.11 Example of data in a spreadsheet event file (in seconds) prepared by the HSC PAL software.

2.4.2 Breaks in sitting

The number and frequency of interruptions ("breaks"), defined as the number of transitions recorded from "sit/lie" posture to "stand" during waking time were counted using the activity profile (summarized by hour) generated by the activPAL™ Professional Research Edition software (Version 5.8.2.3)). This analysis does not require use of the HSC PAL analysis software. For each child, the activity files (summarized by hour) (Figure 2.8) of all monitoring days were printed and the data organised in tables in Excel files for further analysis. Only transitions from sit/lie to stand (u) were counted and not stand to sit/lie (d) transitions (Figure 2.8). Breaks in sitting are quantified as a numerical value (i.e. the number of breaks).

2.4.3 Sitting bouts

For each individual, we calculated the number and duration of each individual sitting bout, defined as duration in seconds spent in "sit/lie" ending in a postural transition (96). The number and duration of sitting bouts (sit/lie) were quantified using HSC PAL analysis software (version 2.14) (102;208) (Figure 2.9). The number of the sitting bouts was calculated by sorting of the data by the colour and then counting the number of sit/lies periods (figure 2.12). Sitting bouts are also characterised by a duration.

In this research we used accumulation curves (96) and a Fragmentation Index (249) to encapsulate the length of individual sitting bouts and their distribution . Accumulation curves (Lorenz curves) characterize how an individual aggregates their sitting time (96;250) , and relate the amount of a particular measure – here time - accumulated in bouts shorter or equal to a given length.

The accumulation curves were generated as follows: for all monitoring days, the number of sitting bout was counted and organised in a table according to their length (0, 1min (60s) length, 2 min, 3 min......up to 90 min) for each child. The sum of all sitting bout was calculated, then the cumulative percentage was calculated and represented in the Scatter curve using Microsoft Office Excel 2007. From the graph the 50% and 90% sitting bout lengths were calculated.

These curves can be reduced to a single metric at any point along the curve, but the 50% sitting bout length and 90% sitting bout length have been suggested to be the most interesting points (250). 50% represents the middle of the data (the typical sitting bout length) and 90% representing encompasses 90% of sitting bout length, and therefore provides a representative value of the longest non-exceptional (i.e. excluding any particular extreme values) sitting bout length.

The Fragmentation Index (calculated as the number of sitting bouts/total sitting time measured in hours) (249) is a metric that summarises information about breaks and the accumulation curves in one single metric. The Fragmentation Index (with units of number of bouts/total sitting hr) normalises the number of breaks in sitting by removing the influence of total sitting time, and provides a simple single measure of whether an individual accumulates their sitting time in many short bouts or in a smaller number of longer bouts (249). A higher Fragmentation Index indicates that time spent sitting is more fragmented, and, therefore, the sitting bouts are shorter, e.g. if a participant was measured for two hours and sat uninterruptedly during this time the Fragmentation Index would be 0, while, if there were a break every 15 min, the Fragmentation Index would be 8. Both accumulation curves and the Fragmentation Index have been used to characterise sitting behaviour in adults (96;249).

Figure 2.12 Example of spreadsheet sorted to calculate the number of sit/lie bouts and the total sitting time, in this example (from 8:37 am to 8:46) the number of bouts was 22 and total sitting(sit/lie) time was 2262.3s (37.7 min).

2.5 Ethics Statement

The University of Glasgow Medical Faculty Ethics Committee approved all the studies involving human subjects. Parents gave written informed consent to participation, and, if possible, the children assented to the individual study procedures.

The researcher obtained Disclosure Scotland approval before the start of the experimental work.

2.6 Statistics

Statistical analyses and calculations were conducted using the Minitab statistical software version 16.1 (State College, PA, USA) and Microsoft® Office Excel 2007. All variables were checked for normal distribution. Results were presented as mean (SD) for normally distributed data, and as median (range) for data that were not normally distributed. For all tests, significance was taken at $p = 0.05$.

Chapter 3

The influence of minimum sitting period of the activPAL™ on the measurement of breaks in sitting in young children

3.1 Introduction

As yet, there has been little research into the possible health consequences of variations between individuals in breaks in sitting time in children. This is largely because there has been a lack of practical, objective, and validated methods suitable for measuring sitting time and breaks in sitting in free-living children(251).

There are two studies that use the Actigraph to determine breaks in sitting. Kwon et al. (88) and Mitchell et al. (107) both reported, in longitudinal studies of older children and adolescents, that Actigraph determined breaks in SB decline with age.

However, at present there is little evidence of the accuracy of the Actigraph for measurement of breaks in sedentary behaviour (107). Concurrent validity of the activPAL™ (against the Actigraph) for group-level estimates of total time sitting (sedentary) time has been established for pre-school (252) and older children (253) . Further, criterion validity (against direct observation) of activPAL™ measurements of time spent sitting was also shown to be high in our previous study of pre-school children (208). However, evidence of criterion validity of the number of breaks in sitting is less clear, and may depend on activPAL™ settings, particularly the MSUP setting in the software (208).

At present, the effect of changes in the MSUP on measurement accuracy of both sitting time and breaks in sitting time is unknown and has not been explicitly investigated. Details of how the MSUP can be changed in the analysis software were described in Section 2.2.2. The two studies to be described in this chapter, the experimental study (Study 1a) and the study of data collected in free-living young children (Study 1b), systematically investigated the effect of changing the MSUP setting.

Two experimental approaches were used. In the first study (Study 1a): the MSUP was varied systematically in an in vitro lab experiment to investigate whether any effects of the MSUP on the measurements of sitting behaviour, breaks, and sitting bouts were evident. In the second study (Study 1b); the findings of the

experimental study were replicated in an in vivo sample using previously collected data from young children.

It is not yet clear how best to use devices such as the activPAL™ to characterise sitting behaviour in young children, including how best to define and measure components such as breaks in sitting. More generally, it is not yet clear what are the best summary measures for characterising and measuring features of sitting behaviour, such as transitions or breaks. Chastin et al. (96) and Lord et al. (254) have proposed a number of fundamental metrics for characterising sitting behaviour in adults using the activPAL™ that might be used to summarise data on sitting behaviour. These include:

- Total sitting time as a measure of the "volume of sitting";

- Number of breaks in sitting characterising the absolute number of breaks but giving no information on the length of time between breaks.

- The number and length of sitting bouts represented as a distribution of sitting bouts by accumulation curves and a Fragmentation Index. These measures give information both on the number and length of sitting bouts.

The extent to which these components and metrics are also appropriate for use in children, particularly young children, is untested. In the analysis, we attempted to report the data using the metrics proposed by Chastin et al. (96) and Lord et al. (254).

3.2 Aims

The specific aim was to examine the effect of variations in the activPAL™ minimum time setting on the total time spent sitting, breaks in sitting, and on sitting bouts. As noted, it was planned to explore this first in an experimental in vitro setting. In the second phase, the effects of varying MSUP were investigated in data recorded in free-living young children.

A secondary aim was to characterise sitting behaviour in free-living young children using the measures proposed by Chastin et al. (96) and Lord et al. (254) and to assess whether these measures provided a useful summary of measures for this age group.

3.3 Method

3.3.1 Study 1a

In the in vitro study, the researcher simulated sitting to standing transitions by turning a monitor by hand from a horizontal position to a vertical position manually while it was recording.

Since the purpose of the study was to investigate the effect of varying the minimum sitting time in the software on the measurement of breaks a series of horizontal to vertical transitions were recorded on one activPAL™ device. It was not judged necessary to use more than one monitor since the parameter of interest was a software setting. The purpose of the study was not to investigate whether there were differences between devices in the recording of the events.

The monitor was prepared for recording the data as described in Section 2.2.1. Once the activPAL™ was ready for recording and the flashing green light every few seconds confirmed that the device was recording, the device was moved from a horizontal to a vertical direction manually (simulating a sit to stand transition) by the researcher. The experimental plan was to investigate different sitting bout lengths, from bout length of 1s to bout length of 10s. The researcher simulated 5 sitting bouts for each sitting bout length, i.e. 5 bouts with length 1s, 5 bouts with length 2s, etc., up to bouts with length 10s.

The whole experiment protocol was repeated 4 times for all bout lengths from 1s to 10s, giving a total of 50 transitions, to ensure accurate measurements, particularly at the shorter intervals. Once the data on all the sitting bout lengths had been collected, the data recording was stopped, the activPAL™ was inserted into the docking station (Figure 2.2), and the data was downloaded to the PC

The raw file was reprocessed varying the MSUP time from 1s to 10s MSUP. Changing the MSUP in the activPAL™ software was described in Section 2.5. The number of sitting bouts (sit/lie) was counted using an HSC PAL analysis software spreadsheet (Figure 2.11). The calculation of the number of sitting bouts was described in Section 2.4.3. The information from the activPAL™ monitor was then compared with the true events, and represented in a graph (Figure 3.1).

3.3.2 Study 1b

The data used in this part of the study were collected from a convenience sample of 23 healthy, free-living preschool children in Glasgow, Scotland. Information letters were distributed to head teachers of nurseries (N = 4) and local contacts (mainly colleagues with pre-school aged children). Parents who agreed to take part made an appointment with the researcher, where written consent was obtained and baseline data and anthropometric measurements were completed. Each child was asked to wear an activPAL™ monitor (PAL Technologies Ltd., Glasgow, UK) continuously, 24hrs a day, for between 5 to 7 days. The measurement of sitting variables using the activPAL™ and the variables used was described in Section 2.2. The data for this study had been collected by previous MSc student Anne Martin to compare measures of sedentary behaviour between the activPAL™ and the ActiGraph accelerometer in pre-school children (252). The raw activPAL™ files of the twenty children were made available by Anne Martin to the researcher for the analysis described.

The data reduction and analysis of sitting variables were described in Section 2.3. The following sitting (sit/lie) variables used to characterise sitting behaviour, during waking hours, were:

1. Total time sitting – measured in hours.

2. Breaks in sitting – a single number without any units.

3. Sitting bouts – measured displayed using accumulation curves and summarised by the fragmentation index.

3.4 Statistical Analysis and Study Power

A convenience sample of around 20 children was deemed a priori, likely to be sufficient to characterise differences in the number of posture transitions, as measured between the 1s and 10s minimum time spent sitting settings. A preliminary analysis of 20 sets of paired activPAL™ data (i.e. 10s and 1s data from the same child) showed that the difference in number of posture transitions measured by the 10s and 1s settings were highly statistically significant, and so only those children recruited to the study at that point were included, and no further recruitment took place. Paired t-tests were used to test the significance of differences in variables measured, , total sitting time; number of breaks in sitting, sitting bout length and Fragmentation Index, using the 10s and 1s settings Repeated measures analysis of variance (ANOVA) using Tukey's correction for multiple comparisons was applied to compare the mean values for each MSUP.

3.5 Results

3.5.1 Study 1

The number of sitting bouts (from 1s to 10s length) is represented graphically in Figure 3.1. There were 50 simulated bouts (events), 5 bouts for each length.

The graph shows that the analysis software, as expected, did not count sitting bouts of a shorter duration than the user-defined MSUP in the new posture. From Figure 3.1, for example, the sitting bouts period (SBP) less than 10s were not counted when we used the activPAL™ setting of 10s MSUP, and we can see only the sitting bouts ≥10s in the graph. By way of contrast, when we used the 1s MSUP, all the sitting bouts, length from 1s to 10s, were recorded, similar to the simulated events. However, there were more sitting bouts at 1s than total number of events.
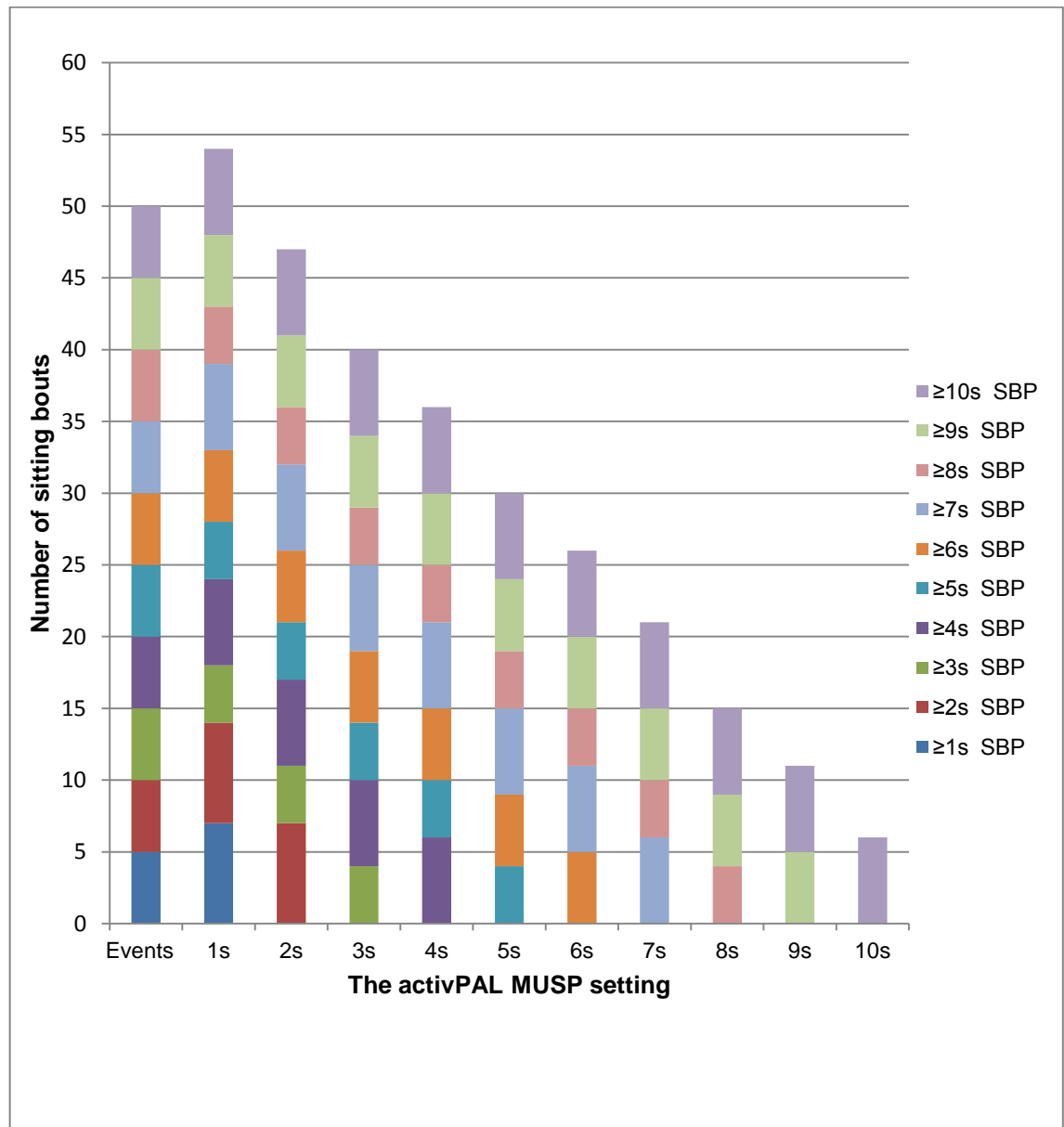
Figure 3.1 The number of sitting bouts counted with varying MSUP activPAL™ settings. Sitting bouts of 1s length in the graph (blue) are shown only when the activPAL™ is 1s MSUP, sitting bouts ≥2s and ≤ 3s in the graph are shown in brown and only appear with an activPAL™ settings of either 1s MSUP and 2s MSUP, ...etc. With a setting of say 10 s, no bout less than 10s duration is counted. The activPAL™ was moved manually.

3.5.2 Study 1b

3.5.2.1 Characteristics of Study Participants

Of the 23 children recruited to the study, 3 children wore the monitors less than 3 days during the study, and were excluded by Anne Martin. 20 children provided adequate data of at least 3 days (208;252), ((9 boys and 11 girls; mean age 4.5) (SD 0.7)); mean height 107.7 cm (4.9), mean weight 19.6 kg (3.9) and mean BMI 16.6 kg/m² (2.0). The mean z-scores were 0.24 for height, 0.60 for weight, with a median z-score 0.16 for BMI. Mean (SD) monitoring time was 3.8d (0.7), 22.3hr (1.5) per 24hr period, of which a mean of 11.9hr (1.0) was in waking hours. Missing data (where monitor was removed because of swimming, bathing/showering, or monitor was not reattached according to the parental record) accounted for a mean of 5.1% (SD 3.4) of total monitoring hours.

3.5.2.2 Breaks in sitting in free-living children

A plot of number of breaks in sitting per hour against time during 24 hrs is shown in Figure 3.2 for both day and night hours using 10s vs. 1s MSUP. There was a gradual increase in the number of breaks per hour from morning until afternoon, with a dip after lunch-time and a peak at around 4pm, followed by a decrease in the evening until the child went to sleep. During the night, generally no breaks were recorded from midnight until early morning. However, occasionally a few breaks occurred between 9pm and 12midnight (Figure 3.2).
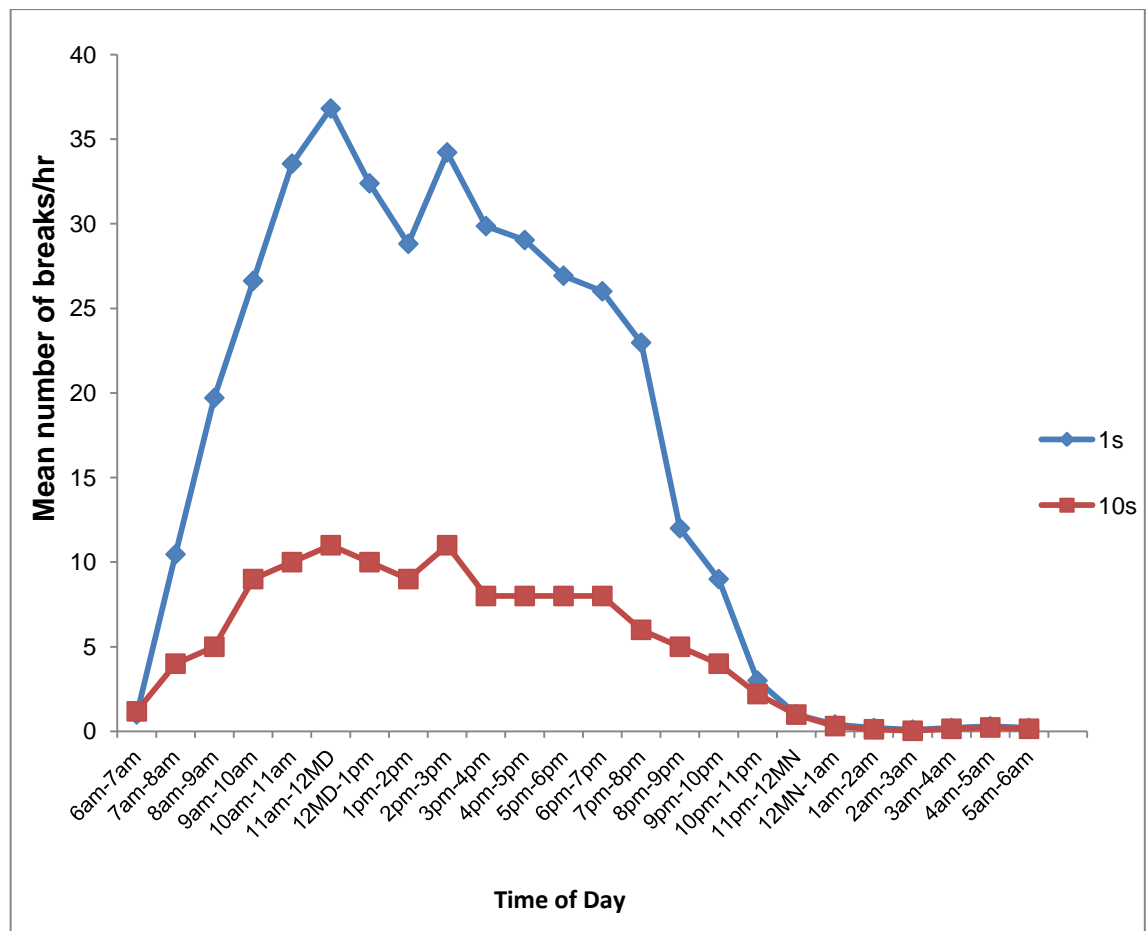
Figure 3.2 The mean number of breaks in sitting/hr over a 24hr day using minimum time to define new position settings of 10s and 1s in free living children (n=20).

In light of the above, the rest of the analysis was restricted to analysis during waking hours (from the first sit-to-stand transition in the morning until 2100, as defined previously in Section 2.3.2).

Using a minimum activPAL™ sitting/upright period of 10s, the mean (SD) percentage of waking time spent sitting was 52.3 %(6.2). The total sitting time was 6.2 hrs, (1.0) during waking hours (11.9 hrs (1.0)). The total number of breaks in sitting during waking hours was 109 (18), giving a mean number of breaks of 8 (3) per hr. Using a 10s MSUP, around 90% of sitting bouts during waking hours were ≤8min (1.5), and the mean (SD) Fragmentation Index (number of bouts/total sitting time (hr)) during waking hours was 19.3 (3.7) (Table (3.1)).

3.5.2.3 The difference in estimated sitting time and breaks in sitting using 10s vs. 1s MSUP

Using the measures advocated by Chastin et al. (96), the measures of sitting time during waking hours with the different MSUP settings are shown in Table 3.1.

There were no significant differences in the mean sitting time when expressed either as total time measured in hours or as a percentage (6.2 hrs (52.3%) vs. 6.3 (52.9%), Paired t-test p = 0.45) (Table 3.1).

However, for the number of breaks, number of bouts, bout periods, and Fragmentation Index (number of bouts/sitting hour) there were significant differences as the MSUP was varied. Changing from a 10s setting to a 1s setting for MSUP led to significant increases in: the total number of breaks in sitting (255) vs. 376 (90), p = 0.001); the mean number of breaks per hour 8 (SD 3) vs. 28 (SD 6), p = 0.0001); and the mean total number of sitting bouts ((118 (SD18) vs. 382 (SD 80), p = 0.0001)). The mean number of sitting bouts during waking hours in 10s and 1s is represented graphically in Figure 3.3 for all participants.

Around 90% of sitting bouts were ≤8min using a 10s setting, but were ≤1min using a 1s setting. The Fragmentation Index using a 1s setting was nearly 3 times greater than when using a 10s setting: (61.6 (16.4) vs. 19.3 (3.7), p = 0.001) consistent with more fragmented, and shorter sitting bouts.

Table 3.1 Description of sitting behaviours during waking hours+, mean (SD) for Study 1b (n=20)

	10s setting (Default) Mean (SD)	5s setting Mean (SD)	2s setting Mean (SD)	1s setting Mean (SD)	p-value#
Total sitting time (hr)	6.2 (1.0)	6.3 (1.0)	6.4 (1.0)	6.3 (1.6)	0.90
% Sitting time (defined as sit/lie only)	52.3 (6.2)	52.5 (5.9)	53.5 (5.4)	52.9 (6.3)	0.70
% Sitting time (defined as sit/lie and quiet standing)	80.1(8.3)	80.3 (4.6)	82.1 (3.9)	81.5(8.9)	0.50
Number of breaks (sit to stand)/hr*	8 (3)	14 (2)	21(4)	28 (6)	0.00
Total number of breaks in sitting (transitions)*	109 (18)	173 (43)	278 (78)	376(90)	0.00
Number of sitting bouts[&]	118 (18)	182 (28)	289 (52)	382 (80)	0.00
50% sitting bout length	80.0s (14.7)	55.0s (4.2)	50.0s (4.2)	42.0s (7.7)	0.00
90% sitting bout length	8.0 min (1.5)	6.0 min (1.1)	3.0 min (1.0)	1.0min (0,2)	0.00
Fragmentation Index	19.3 (3.7)	29 (5.0)	46 (9.0)	61.6 (16.4)	0.00

+ Waking hours were defined as "From the first sit to stand transition in the morning to 9pm" *Calculated from activity profile summarised by hour using activPAL™ Professional Research Edition (Version 5.8.2.3).

[&]Calculated using activPAL™ HSCPAL analysis software (version 2.14). The PAL files generated by the activPAL™ software were imported into HSC PAL analysis software (developed by Dall and Granat).

#Repeated measures analysis of variance (ANOVA).
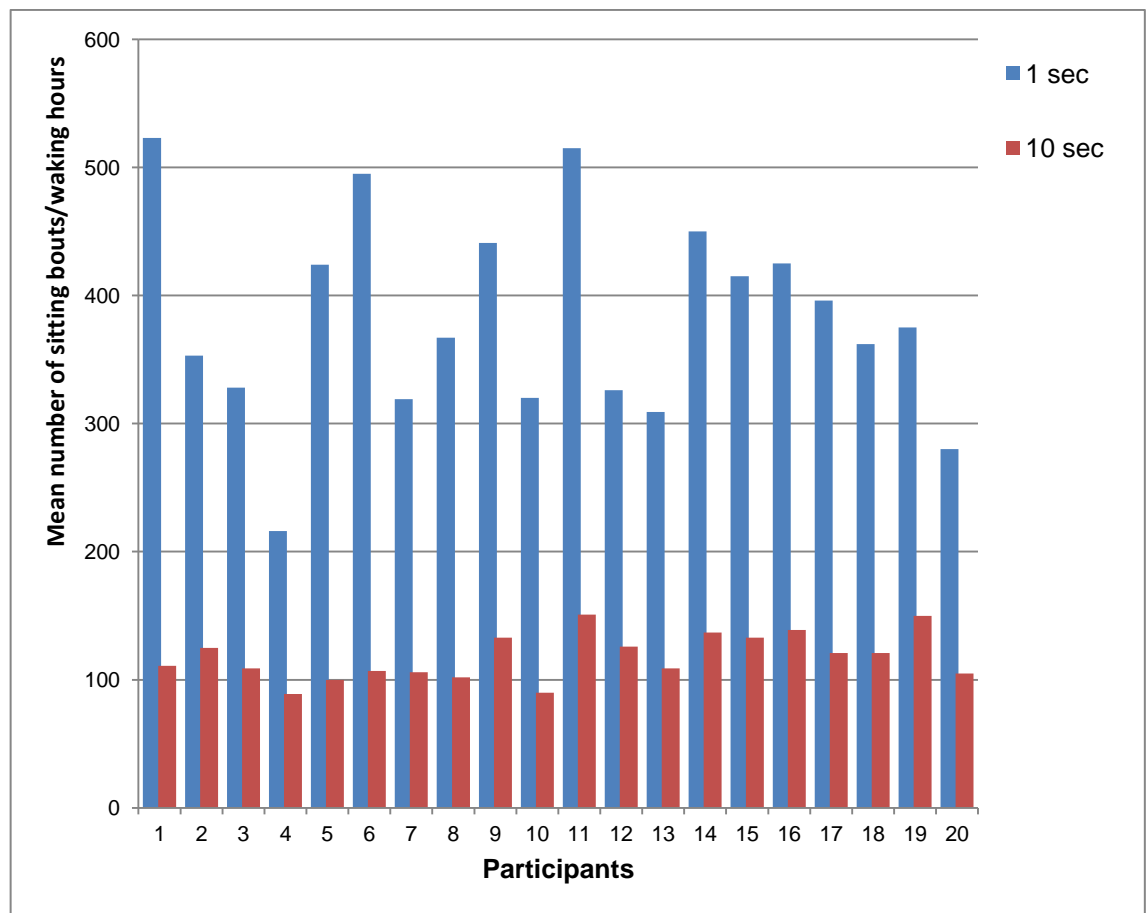
Figure 3.3 The mean number of sitting bouts during waking hours with 10s and 1s minimum time to count new position setting (n = 20).

3.5.2.4 Inter-individual differences in the pattern of accumulation of sitting bouts

In addition, inter-individual differences in the pattern of accumulation of sitting bouts were observed. An illustrative example from 2 children is shown in their Lorenz curves (Fig 3.4). These two children were selected because their total time sitting for both children was similar at 53% and 55% of 10.2 hrs (0.9) and 10.7 hrs (1.0) waking hours respectively; however, the accumulation of their bouts was different: using a 10s MSUP, 90% of sitting bouts were ≤6 min in child 1, and ≤15 min in child 2, and about 50% sitting bouts were ≤55s in child 1, and ≤75s in child 2. Moreover, child 1 had a Fragmentation Index of 17.9 (5.2) and 82.5 (20.0) with 10s and 1s settings respectively, and child 2 had a Fragmentation Index of 15.8 (4.2) and 37.3 (10.2) with 10s and 1s respectively.
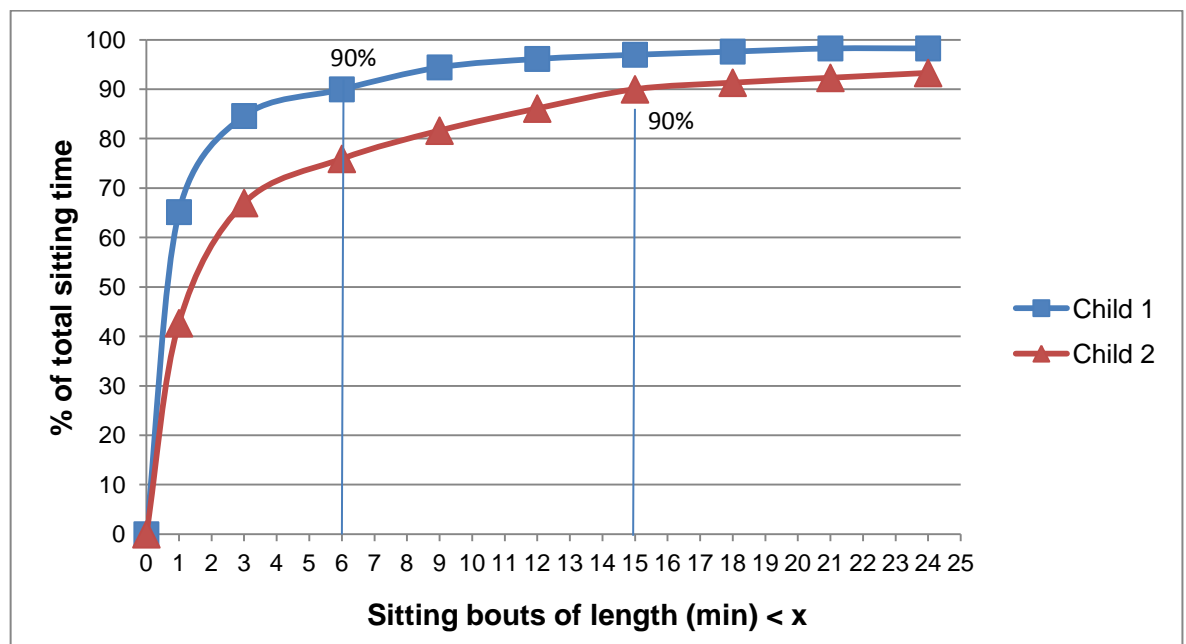
Figure 3.4 Illustrative example of Lorenz curve of sitting bouts from 2 children using an MSUP setting of 10s setting. Despite similar sitting times, these two children show quite different patterns with one child have many shorter periods of sitting.

3.6 Discussion

3.6.1 Main findings and study implications

The result of this experimental study showed that the number of sitting bouts (reflecting breaks in sitting) was affected by varying the activPAL™ MSUP setting. Only breaks in sitting lasting longer than the set user defined MSUP were counted. For example, when a 10s MSUP setting was used, all the bouts less than 10s were not recorded (Figure 3.1). All the sitting bouts lengths were recorded when the 1s MSUP were used, but the total number was more. This may be because when the 1s setting was used, the activPAL™ became very sensitive, and some postures, not sitting, were recorded as sitting bouts.

Since children's movements is considered to be more interrupted and sporadic than adults, and the sitting to standing transition may be quicker in children (102;208), changes in the activPAL™ MSUP setting are likely to have a greater systematic effect on measures of sitting behaviour components in children.

In the second study using data from free-living young children we confirmed that MSUP changes do affect the results. In the twenty young children, we found that varying the activPAL™ MSUP setting had only a negligible and non-significant (p = 0.9) impact on measurement of total time spent sitting. However, and in contrast, for breaks in sitting there was a significant difference (p = 0.0001), varying systematically with the setting used (Table 3.1).

If very short sitting bouts are excluded, their contribution to sitting time may not be great. From Table 3.1, the effect seems to be of the order of around 0.1 hr. However, while the total duration of short sitting bouts may contribute to total sitting time, it has a substantial impact on both the number of breaks and the number of sitting bouts. From Table 3.1, this is exactly what was found. This means that where the measurement of breaks in sitting outcome is the target outcome, then the precise choice of MSUP setting will have a critical impact on the target measure. Studies of breaks should therefore always report the MSUP setting. Results can only be compared if the MSUP is known and essentially identical.

Study 1b also shows that important features of sitting behaviour in young children can be captured objectively by a few fundamental metrics, as proposed by Chastin et al.: volume of sitting (total sitting time); number of breaks in sitting; and the distribution of sitting bouts as represented by accumulation curves and a Fragmentation Index (249;250). The fact that sitting behaviours can be characterised objectively by a few simple measures means that comparative studies investigating the longer-term health effects can now be undertaken in children using these measures.

This is the first time the effect of varying the activPAL™ MSUP to define a new posture setting on measurements of total time spent sitting and breaks in sitting has been studied, either in vitro or in vivo. It is clear that a variation of the MSUP length has affects in both laboratory conditions and free-living conditions).

3.6.2 Comparisons with other studies

Healy et al. (42) previously reported in adults that increased breaks in sedentary time (resulting in short bouts) are associated with better metabolic health, a relationship that was independent of total sitting time. It is, therefore, of interest to see how the results from other studies in children and adolescents compare.

As noted, previous studies have not examined the effect of the activPAL™ MSUP to define a new posture on measurements of sitting time and breaks in sitting. It is therefore of some interest to compare the data of young children to published data.

The average number of breaks in sitting during the day reported in the present study, about 8/hr if the default 10s MSUP setting is used, is greater than previously reported in adults (median 3 per hour) using the activPAL™ (256). A study (100) of adolescent girls with the activPAL™, using a 15s epoch file, found that the mean number of breaks in sitting behaviour was around 5 per hour, with a mean number of sitting bouts around 5 per hour (100). This is less than the number observed in the present study, with around 8/h and 10/h, respectively.

A recent longitudinal study by Kwon et al. (88) described changes in the frequency of breaks in sedentary behaviour from ages 5 to 15 using a pragmatic definition of ≥100 Actigraph cpm to define a break. They found the average daily number of sedentary breaks during waking hours was 525 in children age 5, and 285 by age 15 (88).

In contrast, the two published activPAL™ studies in young children, this study, and one in adolescent females (100), showed a much lower number of breaks during waking hours observed than the number reported by Kwon et al. (88) using the Actigraph (about 40 breaks per hour). The differences between the two studies might reflect methodological differences from using different monitors. The Actigraph is not designed to differentiate between sitting and standing (211).

In free-living adults, Chastin and Granat, using the activPAL™ with a 10s MSUP, found that the mean sitting bout length was 45 min (96). Harrington et al. noted the mean length of sitting bouts in adolescent females using the activPAL™ was 9.8 (0.2) min (100). These authors used a customised MATLAB programme to process the activPAL™ data output files, and their analysis used 15s epoch files rather than an event based approach (100).

We found - using a 10s minimum sitting time for purposes of comparison - that the majority of sitting bouts for the young children in the present study lasted ≤8 min, suggesting that the children studied predominantly accumulated their sitting time in short bouts.

Studies using objective measures of the Fragmentation Index are non-existent in children and scarce in adults. A recent study into 30 healthy adults (using the activPAL™ continuously over 7 days) found that the mean Fragmentation Index [bouts/sitting time (including sleeping time) (hr)] in men was 2.6 (0.8) and 3.3 (0.4) in women (249). In the present study, the mean (SD) Fragmentation Index (again using the default 10s MSUP for comparison) was much higher, 19.3 (3.7). Our present study is not directly comparable because we excluded sleeping time, where subjects would be expected to have no postural transitions. A preliminary reanalysis of 3 subjects in the present study, chosen at random,

showed that even including sleeping time the Fragmentation Index is about 3 times greater than that reported by Chastin et al. (249).

Shorter (than default) MSUP's in pre-school children seem to be required to identify the rapid sit-stand transitions (breaks in sitting) which they make, and so are more sensitive to sit-stand transitions than the default setting. In older children and adolescents the time taken for sit-stand transitions may be greater, and it is possible that longer MSUPs would be appropriate for measuring sit-stand transitions, but there is currently no clear evidence on this. For measurement of sitting time, it would seem that the choice of MSUP is relatively unimportant.

The comparisons with previous studies are somewhat unsatisfactory because it is not always clear what the MSUP setting has been. However, our evidence suggests that settings less than the default of 10s will result in greater numbers of sedentary bouts being recorded. Even using the longer default setting, our evidence suggests that young children have a much more fragmented sitting time, with a pattern of shorter sitting bouts interrupted by more frequently by breaks. The true effect may be even greater if settings less than 10s MSUP are correct.

Because of its impact on the measurement of breaks in sitting and other measures, such as the fragmentation index, the present study suggests that more attention must be paid to this instrument setting. It seems intuitively likely that the most suitable setting for measurement of breaks in sitting time may vary with age. We would hypothesise that children can transition to a new posture more frequently than adults, and the optimum setting for measurement in breaks in sitting may lengthen as subjects get older. We suspect that it is likely that empirical studies using the activPAL™, or other similar event-based monitoring systems, will in future be required to define the best setting for minimum duration of sitting for each age.

3.6.3 Study strengths and limitations

This methodological study shows that there is no difference in sitting time with any setting. The shorter the MSUP setting, the more breaks/transitions are observed in young children. It included no visual recording or observation of the

actual behaviour and cannot therefore determine the most accurate setting by comparing observed behaviour with changes measured on the Actigraph.

It is true that the optimum setting may change as children get older. However, the biological implications of this are at present unknown. It is known from a few other studies in young children that posture changes from sitting to standing and vice versa are occurring more quickly than 10sec (102;208). Faster than, but what is the implication of this for the future. Further this study does not show whether it is better to use a single value or a range of settings for different ages. Only further studies that compare health outcomes with differences in setting will be answer this point. However, it is at least plausible to hypothesise that more frequent postural transitions in younger children may have a significant impact on the development of the autonomic and cardiovascular system with long term health consequences.

The present study does not assess the biological importance of sitting time or fragmentation. However, that was not the aim of the present study. Evidence addressing some of the methodological issues around how best to measure sitting time in young children, and of the establishment of accurate yet simple and objective measures for characterising sitting time and fragmentation, will be fundamental to future studies which try to relate these measures to health outcomes, and essential for evaluation of future intervention studies.

3.6.4 Conclusion

This study has established that the setting of the MSUP to define a new posture has a significant impact on measurement of breaks in sitting in young children, but not the measurement of total sitting time. Sitting behaviour can be usefully characterised using measures of volume, numbers of breaks in sitting behaviour and the pattern of bout length in young children.

From this study and the limited published literature, there appear to be important differences between children and adults. Children have much more fragmented sitting times with shorter sitting bouts - more interrupted by breaks - than in adults. Further studies will be needed to confirm these findings, and to define the most appropriate minimum duration before registering a transition.

Chapter 4

Comparing objective sitting time (observed) with measured sitting time at different MSUP settings

4.1 Introduction

In adults, the available evidence points to more frequent changes in posture being generally beneficial for health (118;257). Similar evidence is limited in children and youth or not yet available for young children.

The activPAL™ and Sampling The activPAL™ is an event-based monitor sampling at 10Hz (10x per second). This gives it the potential to capture very frequent changes in posture. However, it is not clear that postural changes occurring at a frequency of even $\leq$ 1 HZ (i.e. 1 change per sec) are likely to have any physiological meaning.

In order to screen out very short events, the activPAL™ software includes an algorithm that only counts events longer than a specified duration. This setting is acting as a low-pass filter passing low-frequency signals but attenuating signals with frequencies higher than the cut-off frequency. This is set by default at 10s MSUP (i.e. ≥10s of sitting/lying or upright data is needed to register as a new sitting/lying, or upright, event).

In effect, this software setting determines the minimum period to define a new posture, such as sitting (102;208). In many published studies, this setting has been left at the default value of 10s, as per the manufacturer's specifications (258).

However, in a previous study into pre-school children, the researcher changed the MSUP (which can be varied within the activPAL™ software from 1s to 100s) (Figure 2.3) to 1s because posture transitions appeared to be more rapid than the 10s default in young children than in adults (102;208). Using this 1s setting, they found that the activPAL™ provided accurate relative rank-ordered assessments of breaks in sitting, but significantly overestimated the number of breaks in sitting when compared to direct observation (102;103).

It is easy to envisage that the time required to transition from one posture to another, e.g. sitting to standing, might be different at different ages. A young child would be expected to change posture very quickly, but an elderly person might take much longer. However, at present, the optimum activPAL™ setting of

a MSUP to define a change in posture for measurement of sitting time and breaks in sitting is not known, either for early childhood, later in childhood, or adult life.

4.2 Aim

The aim of the present chapter was therefore to determine the criterion validity of different minimum event duration settings using direct observation as the criterion method.

4.3 Methods

The study group was a convenience sample of 32 pre-school children (mean (SD) age, 4.1y (0.5)) recruited from nursery schools in Scotland who were videoed for an hour while playing freely at nursery while wearing an activPAL™ monitor by Dr. Gwyneth Davies. Data analysis was performed on children with a complete data set for activPAL™ and direct observation outcomes. The study is described in detail elsewhere (102;208), but, in brief, each child wore an activPAL™ monitor and simultaneously was filmed for one hour during their usual activity in nursery. A second-by-second direct analysis of the video was then used to count the number of breaks in sitting time.

The raw activPAL™ files, which were saved using a 1s MSUP setting, and coded direct observation files of thirty children were obtained from Dr. Gwyneth Davies by the researcher. The coded direct observation files were Excel files that contained analysis of each second of direct observation data summarized by Dr. Davies as sit, lie, stand, walk, ''other,'' or off screen for each child. The original video files were not reviewed. The raw activPAL™ files were reprocessed by the researcher using a MSUP of 2s, 5s, and 10s and for each child. Changing the MSUP in the activPAL™ software was described in Section 2.2.2. The total sitting time and the number and duration of sit/lie periods was calculated from direct observation files, and was compared with the activPAL™ analyses using the varying settings.

4.4 Statistical Analysis and Study Power

A Bland-Altman analysis (259) for assessing agreement between two measurements was carried out.

Bland-Altman plots (259) are standard ways of examining agreement between two methods which purport to measure the same thing. They illustrate: the mean difference between the two methods (the error or bias in the case of a new method being validated against an established method); the range of differences between the two methods (range of errors for each individual); whether or not the error varies systematically with the size of the variable being measured.

The limits of agreement between the number of sitting bouts during direct observation (criterion method) vs. sitting bouts calculated by the activPAL™ using different MSUP activPAL™ settings (1s, 2s, 5s, and 10s MSUP) were set at a mean difference +/- 1.96 x standard deviation (SD). The graph for the 1s comparison has previously been published (102). ANOVA was applied to compare the total sitting time during direct observation and the total sitting time using different activPAL™ settings. The pattern of accumulation of sitting bouts by direct observation data and activPAL™ data with different settings (1s, 2s, 5s, and 10s) was represented by accumulation curves (i.e. cumulative percentage plots) as described earlier in the methods in Section 2.3.3 (96).

4.5 Results

4.5.1 Study Participants

Thirty preschool children completed simultaneous activPAL™ and direct observation monitoring. Two children were excluded by Dr Davies because they had incomplete data; they wore the monitor for a very short period. The thirty children (10 boys and 20 girls; mean age 4.1yrs (0.5), mean height 105.1 cm (5.1), mean weight 18.7 kg (3.8), with a mean BMI 16.8 kg/m2 (2.1)). The mean z-scores were 0.64 for height, 0.79 for weight, and 0.60 for BMI. A total of 16,167s (14.2%) was 'off-screen' time from 113,917 total measured seconds for

the 30 children (102;208). Total time spent sitting: direct observation vs. different MSUP activPAL™ settings.

Combining data from all participants (n=30), the total time spent sitting during direct observation were compared with the total time spent sitting using the activPAL™ setting 2s, 5s, and 10s MSUP. The total time spent sitting was 12.5 hr during direct observation, and 11.3 hr with 1s MSUP (102). With 2s, 5s, and 10s the total sitting time was 11.4 hr, 11.2 hr, and 11.3 hr, respectively. There was no significant difference (p=0.70) between the total sitting time during direct observation and the total sitting time using difference the activPAL™ setting.

4.5.2 Number of sitting bouts: direct observation vs. different MSUP activPAL™ settings

For bouts of sitting, the average number of bouts per hr using direct observation was compared with bouts measured simultaneously using the activPAL™ using 2s, 5s, and 10s MSUP respectively. Figure 4.1 shows Bland-Altman plots comparing the different numbers of sitting bouts during direct observation vs. different MSUP of 1s, 2s, 5s, and 10s on the activPAL™ for each child (n = 30) are shown.

From inspection of the graphs, it can be seen that the use of a 2s setting for activPAL™ MSUP minimised bias and showed no significant difference relative to direct observation (limits of agreement -14 to +17 bouts per hr, mean difference 2, paired t-test p = 0.20). However, the 5s and 10s settings underestimated the number of sitting bouts as measured by direct observation (for 5s limits of agreement -23 to 8, mean difference -7, and for 10s limits of agreement -29 to 4, mean difference -12.57, paired t-test p = 0.001, respectively). Further, the differences at 5s and 10s are clearly not random.

While the bias is much smaller with a 2s setting, the limits of agreement are quite wide, and of similar magnitude to the other settings. This means that the average with a 2s setting will be more accurate, but for any individual the errors with 2s will be nearly as large as for the other settings.
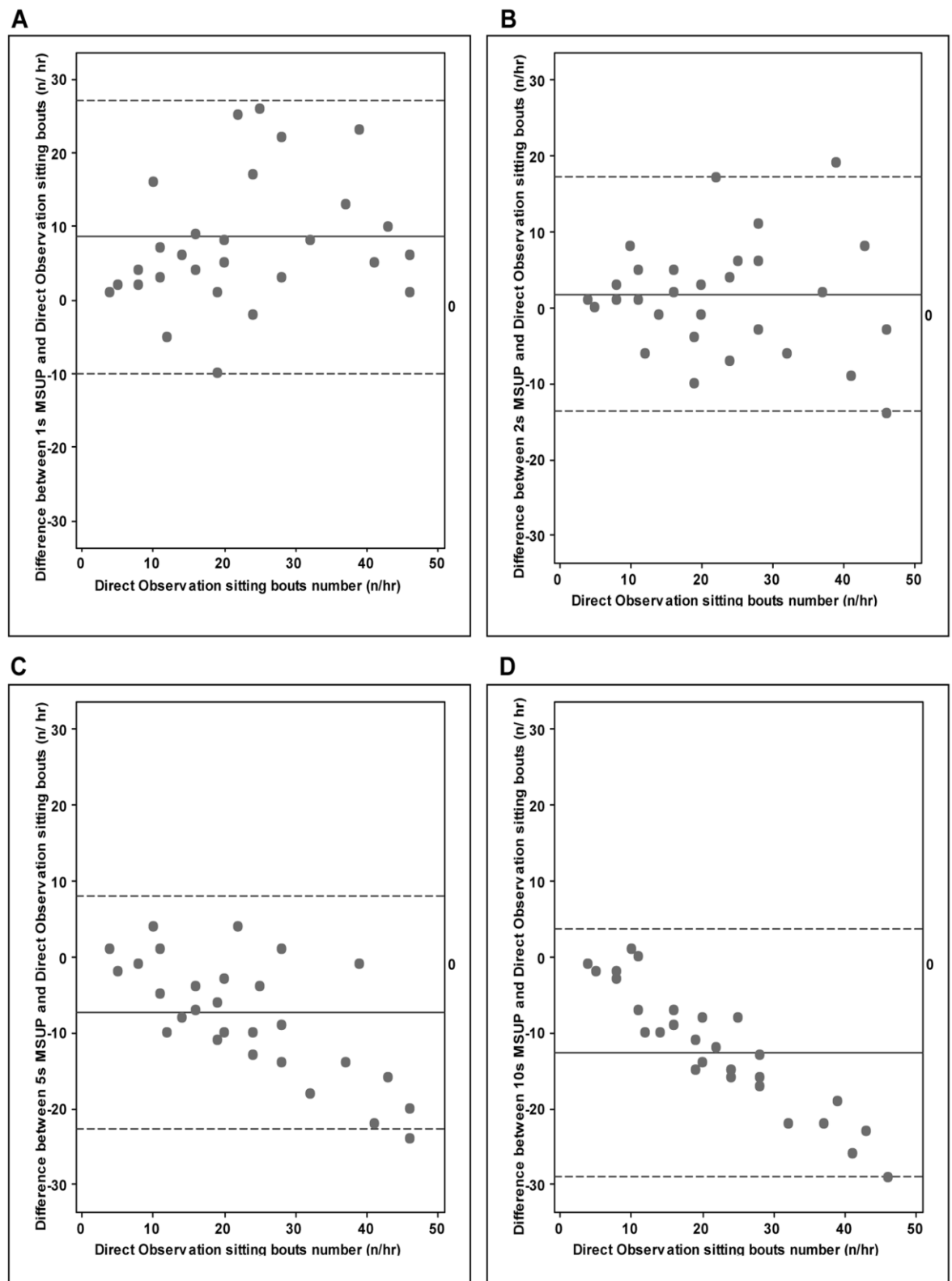
Figure 4.1 Individual Bland-Altman plots comparing the difference in number of sitting bouts during direct video observation (direct observation) with the number of sittings bouts measured by the activPAL™ with different activPAL™ settings for MSUP (1s – diff1 (A), 2s – diff2 (B), 5s - diff5 (C) and 10s - diff10s (D). Data for 1s taken from Davies et al. (102). Direct observation is considered the criterion or gold-standard, and is used on the x-axis. Mean bias is represented by a solid line, 95% limits of agreement by dashed lines.

4.5.3 Pattern of accumulation sitting bouts: direct observation vs. different MSUP activPAL™ settings

Figure 4.2 shows the pattern of accumulation of sitting bouts during direct observation with 1s, 2s, 5s, and 10s MSUP. The accumulation of sitting bouts with 2s was very close to the pattern during direct observation. 90% of sitting bouts were identical (at ≤2 min) for both direct observation and from the activPAL™ using the 2s MSUP.
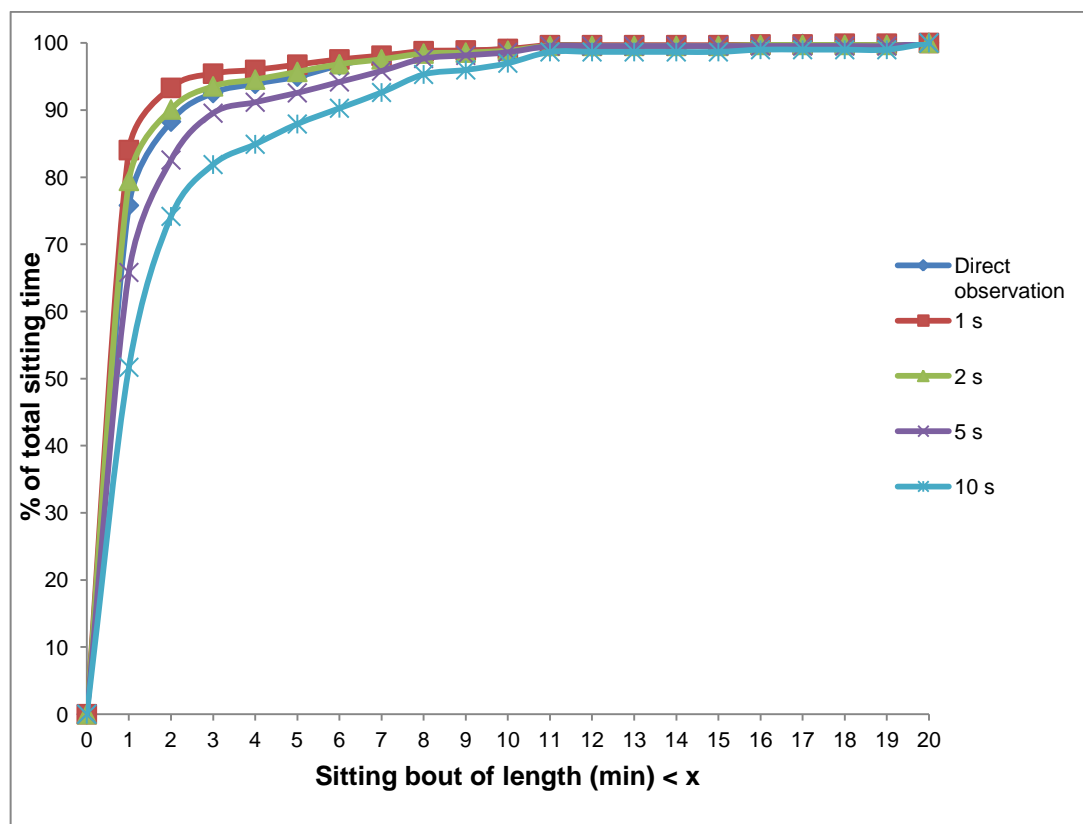
Figure 4.2 The pattern of accumulation of sitting bouts during direct observation, using MSUP settings of 1s, 2s, 5s, and 10s. Study 2 (n=30). The numbers on the x-axis are cumulative – hence the y intercepting at a particular x value represents the number of sitting bouts occurring shorter than or equal to a given x-axis value.

4.6 Discussion

4.6.1 Main findings and study implications

This is amongst the first study to investigate systematically the most appropriate MSUP for young age children (102). The study showed that the differences in MSUP measures have an impact on the accuracy of the measurement of breaks in sitting. The result is that a widely used, default setting of 10s for the activPAL™ appears unsuitable for quantification of breaks in sitting in young children, in whom a MSUP of 2s will provide much higher accuracy with minimal bias. For young children, a 2s MSUP may, therefore, be the most suitable choice.

In our participants, using a 2s MSUP, the mean (SD) volume of sitting in Study 1b was 6.4 hr (1.0) during waking hours, the mean (SD) number of breaks in sitting around 21/hr (4), the Fragmentation Index 46 (9.0), and 50% of sitting bouts were less than 50.0s (SD 4.2) (Table 3.1). This data points to a pattern of a considerable amount of sedentary activity interrupted by frequent short posture changes.

In this analysis, the direct observation coded data were from a previously peer reviewed, published study (260). The analysis of the direct observation in this study was not checked but full details of the methodology are given in the original publication(260).

4.6.2 Comparisons with other studies

To date, no previous studies have determined validity of different minimum event duration settings. Indeed, and in general, other studies have not commented on what setting was used to define MSUP. As an example, Lyden et al. (211) in a recent activPAL™ validation study (against direct observation) of 13 free-living adults monitored for approximately 10 consecutive hours on 2 separate days [4M: 9F; mean (SD) age 24.8 yrs (5.2)] reported that the activPAL™ was a suitable tool to measure breaks in sitting in this older age group, with 5.1 (range 2.8-7.1) breaks in sitting per sitting hour (211). This study did not specify whether the default 10s MSUP was used.

4.6.3 Study strengths and limitations

Previous studies of movement in young children and adults, particularly those using the Actigraph monitor, have used an analytical approach based on the analysis of sitting in 15s epochs. In our studies, the activPAL™ HSC PAL software files and the activPAL™ pal files were used in our data analysis. We made no use of the "15s epoch file" in the available activPAL™ software, as this was beyond the scope of this research. Furthermore, the event-based approach used by the activPAL™ provided a more logical basis for capturing transitions than one based on evaluating levels of activity within defined epochs.

4.6.4 Conclusion

In the preschool children we studied, this systematic investigation showed that 2s appears to be the most appropriate MSUP to define breaks in sitting using the activPAL™.

It is probable that the optimum instrument setting for MSUP will be different at different ages. Standardisation of the technical aspects of measurement and of measures to describe sitting time will allow longer term studies of the health effects of sitting behaviours, as well as providing comparable baseline data for intervention studies.

Chapter 5

Sitting Behaviour in Overweight and Obese vs.Non- Obese.

5.1 Introduction

The prevalence of obesity and being overweight has increased worldwide in childhood and adolescence, an increase that has been more dramatic in economically developed countries and urbanised populations (261). Excess weight in childhood and adolescence is associated with an increased risk of physical and co-morbid psychological issues, and cardio-metabolic morbidity, such as diabetes, hypertension, and respiratory disease, both in childhood and later in life (262;263).

The identification of risk factors for obesity is considered the key to prevention. A recent study showed that time spent on sedentary behaviours, such as TV viewing, is one of the factors independently associated with children's obesity risks (264). Current recommendations for school-age children suggest that sedentary leisure time, such as television (TV) viewing and screen-time use (computers and/or video games) should be limited to 2 hrs/d (72). The Australian guidelines for pre-schoolers also recommend the restriction of screen-based sedentary behaviour to <1 hr per day (22).

It is now sometime since Levine et al. (237) reported differences in postural allocation (sitting vs. standing) in a small study that compared 10 obese to 10 non-obese adults. For the study, Levine et al. used specially developed micro-sensors that captured data on body postures and movements in obese and non-obese adults. In this small study, non-obese individuals stood for about 2.5 hrs longer per day than obese participants, while the obese individuals sat for more than 2 hrs longer per day than lean participants (237).

It is not just the amount of sedentary activity in a day but how sedentary behaviour is accumulated (number of breaks in periods of SB – sedentary bouts - and transitions from sitting to standing and vice versa) as well as the length and frequency of sedentary bouts that may influence health risks; for example, more breaks in sedentary time have been associated with lower WC, lower BMI, and reduced metabolic risk factors, independent of the total sedentary time and moderate-to-vigorous physical activity (MVPA) in adults (118;265).

Intervening on the patterns of sitting time may only be useful if patterning is associated with a health outcome. To date, there is a lack of data relating breaks in sitting and health outcomes over time in young children. Other issues such as the temporal patterning of breaks in sitting, for example, differences between morning breaks and say evening breaks have not been considered at all in the literature. Similarly there is a lack of information on seasonal differences and any relationship to climatic variation. Such studies will only be possible if valid measurement methods for quantifying sitting breaks can first be established.

Whether similar differences in posture allocation can be observed between overweight/obese and non-obese children has not yet been investigated. This is of particular interest because patterns of activity in children might be quite different from adults. If similar differences in patterns of activity were present in obese and non-obese children it would be useful and important information for designing future obesity intervention programmes. For example, it might be possible to use feedback on physical activity and sedentary behaviour as a motivational aid to encourage behaviour change. This is important since following the physical activity and sedentary behaviour elements of obesity treatments are problematic for most families (266).

In this chapter, we therefore conducted a pilot study to investigate the differences in sitting behaviour, including sitting time, breaks in sitting, and sitting bouts length between overweight/obese and healthy weight children.

5.2 Aim/Primary and Secondary Objectives

The aim of this study was to complete a pilot study to examine and describe the components of sitting ('sedentary') behaviour in overweight/obese children, and to then compare these components with normal weight children. We were particularly interested in breaks in sitting and the length of bouts of sitting.

In the study, we used activPAL™ accelerometers. Since these are very small and robust they are suitable for studies in young children. Since no activPAL™ data exists for this sort of comparison, our study was a pilot one, aimed mainly at determining the sample size needed for future studies.

5.3 Method

The present study was a pilot observational case-control study. The inclusion criteria used for the study were as follows: children between 5 and 11 yrs with obesity or who were overweight. Obesity was defined as a BMI ≥95th percentile (the cut-off for obesity is a Z-score of +1.64) and overweight as ≥85th percentile (BMI Z-score +1.04) for age and gender – UK 1990 reference charts (243;244).

The study was a pilot study because there was no information on the differences or the variance of the differences in sitting behaviour between groups of young children of different BMI.

5.3.1 The participants' recruitment

We planned to recruit 40 children from a number of sites:

Sites of recruitment for overweight/obese children

1. Via weight management and dietetic clinics

Between May 2012 and September 2012, we approached children who met the above inclusion criteria and their parents attending weekly weight management and dietetic clinics run by a consultant paediatric endocrinologist (Dr. Guftar Shaikh) and a specialist dietician (Jill Morrison) at the Royal Hospital for Sick Children Yorkhill.

The dietician provided the researcher with the list of the children who would be attending the clinical for the following weeks. The list contained only the name of child, date of birth, contact address, diagnosis, date and time of the next appointment. From a review of the list, we identified 20 families whose children met the inclusion criteria at their last clinic review. These families were then approached by the researcher at their next clinic visit; the researcher met the families in a room beside the clinic, after they finished their appointment with the dietician. Out of 20 who were approached, 9 parents agreed to receive preliminary verbal information about the study and a letter of invitation to take part (see Appendix A), given to them by the researcher at the clinic. Of these 9, only four parents indicated an interest and agreed to receive more detailed

information. They were given information sheets (one for the parent and one for the child, see Appendix B), and a consent form (Appendix C), and the four parents, with their children, were willing to participate.

When informed written consent had been obtained from the parents' of the four participants, the baseline data collection and measurements were undertaken. Each child was asked to wear an activPAL™ monitor (PAL Technologies Ltd., Glasgow, UK) continuously, 24 hrs a day, for between 5 to 7 days; data collection has been described in Section 2.2. The parents were also asked to complete an activity log sheets (Appendix D), and to record the date and time of any periods when the monitor was taken off and reattached.

About 4 months from the beginning of recruitment at the clinic, it became clear that that recruitment was very slow and was proving much more problematic than expected. This was mainly due to the small number of children who attended the clinic, which had a high "Do not Attend" (DNA) rate. Since the clinic was a specialist multi-disciplinary clinic it was not anticipated beforehand that the DNA rate would be high. We concluded that additional sources of participants would be required if a target of 40 children was to be reached.

To accommodate these new sources, the original ethics application was modified. The amendments, which involved minor changes in the information sheet's wording and invitation to participate, were submitted to the Ethics Committee for approval, and on 30 October 2012, a substantial amendment was approved.

The following potential additional sources of subjects were identified:

2. Children who had previously attended the weight management and dietetic clinics at Yorkhill run by Dr. Guftar Shaikh and Jill Morrison (specialist dietician) with simple obesity or who were overweight.

A letter of invitation to take part, information sheets about the study, and a consent form were sent by post to 24 parents whose children met the above inclusion criteria. Parents who were willing to participate were asked to complete the consent form and return it in the enclosed stamped addressed

envelope. At this point, we planned to contact them and arrange a meeting to answer any questions, confirm consent, and start monitoring (see the Flow diagram of study recruitment Figure 5.1).

3. Children who were attending the Active Children Eating Smart programme run by NHS Greater Glasgow.

The Active Children Eating Smart (ACES) programme is a 12-week programme for 5-15 yrs olds who are overweight or obese. It takes place in local schools, leisure centres, and/or community venues, and aims to help them to either lose weight or not gain further weight (267).

All the information about the study was sent to the Health Improvement Lead for the ACES programme, Anne Gebbie-Diben. After the programme board had agreed to take part in the study, we then met her and discussed the method of recruiting children. First, the ACES/Active Development Officer - Mr Paul Mclean (who also received all the information about the study) - identified children who fully met the inclusion criteria, and sent their parents the information on the study. Then the researcher met the parents who were willing to participate with their children on the first week of the ACES programme at three different locations (The Glasgow Club Maryhill on 11/2/2013, St Margaret Mary's High School on 11/2/2013, and the Glasgow Club Haghill on 14/2/20013). Overall, 14 children were approached.

4. Children attending primary schools in Scotland.

As controls, healthy weight children that matched the overweight/obese study participants for age and gender were recruited from the local schools.

In this study, three primary schools were involved. One was privately operated (in Glasgow). Permission for study recruitment was obtained from the headmistress of the primary school.

Two others were primary schools run by the Libyan Embassy in London (the Libyan school in Glasgow and the Libyan school in Edinburgh). There are twenty schools across the UK run by the Libyan Embassy in London, three of which are in

Scotland (Glasgow, Edinburgh, and Aberdeen). Libyan schools run during the weekends and teach the Libyan curriculum; these schools are mainly for Libyan children who have a parent studying or working in the UK. The permission for study recruitment was first obtained from the School Department at the Libyan Embassy in London.

The participants' recruitment from the schools was carried out between the middle of November 2012 and the middle of April 2013. Letters of invitation were sent to the schools' head teachers and, after the schools agreed to participate, information sheets (parent information sheet, child information sheet; Appendix A and Appendix B), and consent forms were sent out to the parents/guardian of the school children in Primary One and Two classes.

From the three schools, the researcher met the 47 parents and their children who were willing to participate at the schools (5 parents preferred to meet the researcher at a Clinical Research Facility department at the Royal Hospital for Sick Children). Written parental informed consent and verbal assent from the children were obtained prior to child recruitment. Further verbal information was given to the parents and children at the start of the study.

It was anticipated that most of these children would be of healthy weight and be suitable as controls for children who were overweight or obese. However, it was recognised that some of the children might be overweight or obese.

Exclusion Criteria:

Children with any underlying disease that was likely to affect their physical activity or sedentary behaviour were excluded. For the controls, any children who were overweight or obese were excluded as controls although they were then eligible to be included with the overweight/ obese. The flow diagram of study recruitment is shown in Figure 5.1.
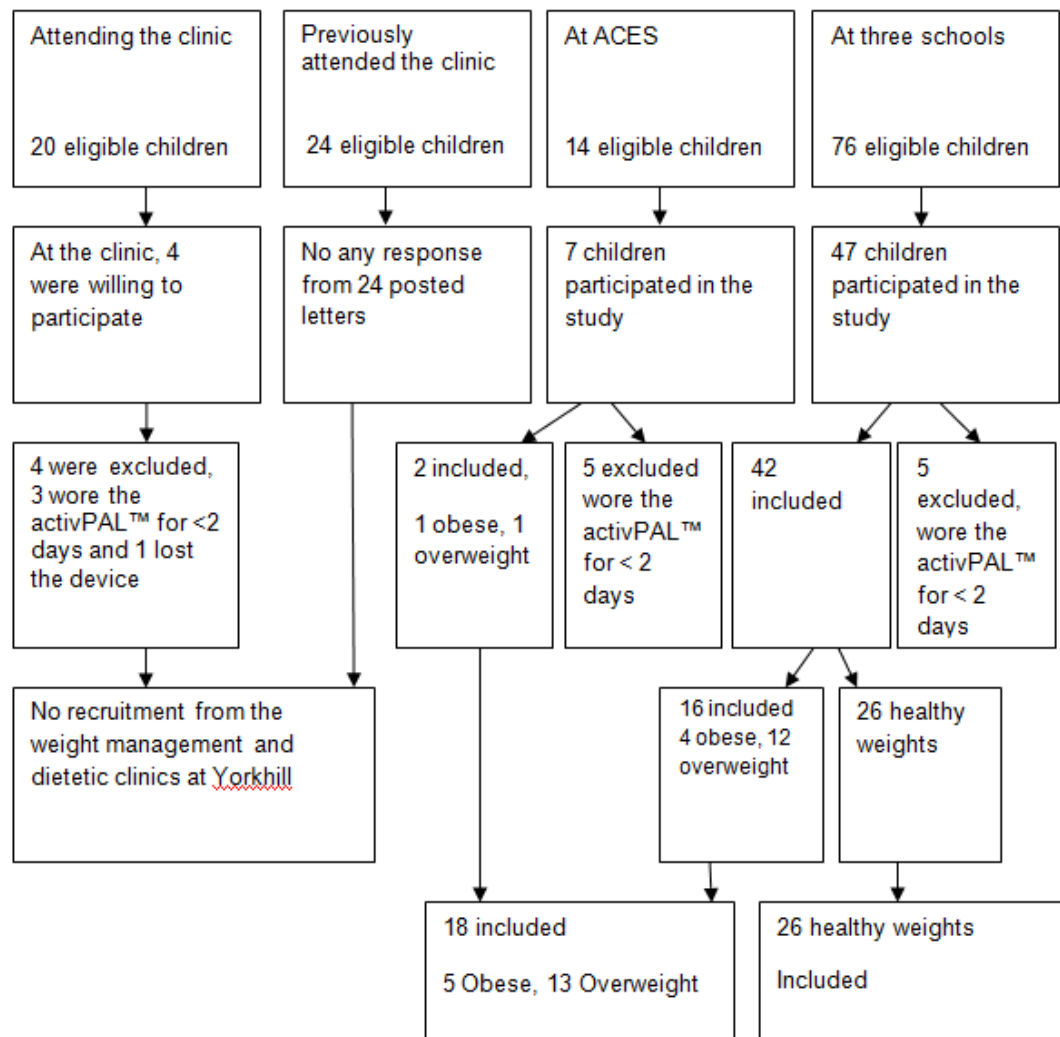
Figure 5.1 Flow diagram of study recruitment

No child had to be excluded because of underlying disease that was likely to affect their physical activity or sitting behaviour.

5.3.2 Measurement of sitting variables using the activPAL™

All participants had their age, height, and weight recorded at the start of the study. The anthropometric measures were described in Section 2.1. Each participant was asked to wear an activPAL™ (Pal Technologies Ltd., Glasgow, UK) monitor for 7 consecutive days, 24 hrs a day. The only times the monitor was to be removed were during periods of bathing or swimming because the monitors were not waterproof. Participants wore the activPAL™ on their mid-thighs. Measurements of sitting variables using the activPAL™ were described in Section 2.2.1. See Figure (2.4).

Parents were asked to record the date and time of any period when the monitor was taken off and reattached in provided activity log sheets (Appendix D). The activPAL™ attachment materials (Tegaderm and PALstickie) - see Figure (2.5) and Figure (2.6) were also provided in sufficient amounts by the researcher.

At the end of 7 days, the researcher collected the activity monitors and activity log sheets from the participants (at the clinic, the school, or at the Active Children Eating Smart programme centres). The data was then transferred into a computer for analysis using special software provided by the manufacturer.

5.3.3 Data processing

The data download process was described in detail in Section 2.2.6. Briefly, the activPAL™ classifies the periods of the recording time in different postures, categorized as lying/sitting, standing, walking, and transitions. By using activPAL™ Professional Research Edition software (Version 7.1.18), the data from all devices were downloaded to a computer for data analysis, and the raw activPAL™ files were reprocessed using a MSUP of 2s for each child, as described previously. For each participant, the total sitting time (sit/lie), the percentage of daily time spent sitting, and the number of sitting bouts (reflecting the number of breaks in sitting and duration of sit/lie periods) was calculated using software (HSC PAL analysis software version 2.14). Data reduction, measurement of sitting variables, and changing the MSUP in the activPAL™ software were described in detail in Section 2.2.2, 2.3 and 2.4.

5.4 Statistical analysis

Sample Size: The study was performed as a pilot study, so it was not possible to determine sample size precisely in advance. We aimed to recruit 18 overweight or obese children, with 18 matched controls for age and sex, giving a total of around 36 participants entered into the study. Based on previous experience with the activPAL™ (208;252), we expected that entering around 40 children would provide reliable activPAL™ data (at least 3 days of data with at least 6 hrs per day (208) from at least 36 children (18 paired comparisons). While this study was undertaken to provide data required for a power calculation for the different components being measured (total sitting time, breaks in sitting time), we thought from previous studies that a sample of around 16-18 paired comparisons might be sufficient to detect significant differences in total time spent sitting between overweight/obese children and non-overweight/non-obese controls.

2-sample t-tests were used to detect differences between the two groups for normally distributed. The 50% and 90% of sitting bouts length were not normally distributed, and thus a Mann-Whitney test was used to determine the significance of between-group differences. ANOVA using Tukey's correction for multiple comparisons was applied to compare the sitting behaviour components of overweight, obese children and healthy weight children. Linear regression was performed between BMI Z score and number of sitting bouts (the variable of most interest) and represented by a graph.

5.5 ResultsCharacteristics of participants

5.5.1.1 Overweight and Obese participants

At Yorkhill dietetic clinics: *overweight/obese children who attend the clinic*: From 9 parents who agreed to receive preliminary verbal information about the study, 4 consented to participate but did not complete the study (3 wore the activPAL™ for less than two days and 1 lost the device).

Overweight and obese children who previously attended the clinic: We did not receive any response from 24 posted letters. Therefore **no children** were recruited from the Yorkhill dietetic clinics (Figure 5.1).

At the ACES programme: from 14 eligible children, 7 children participated in the study, 5 wore the activPAL™ for less than two days and their data was excluded. Only 2 (1 overweight, 1 obese) children completed the study and provided usable data.

At schools: from 76 children, forty-seven from three schools participated in the study. Five children's data had to be excluded (four children wore the device for less than three days and one child lost the device). Sixteen children weighed above the healthy weight for their age (12 overweight and 4 obese), and twenty-six children weighed within the healthy weight range parameters (Figure 5.1). No child was excluded because of an underlying disease likely to affect her/his physical activity or sitting behaviour.

Finally, the total numbers of overweight and obese children taking part in the study who provided adequate data from the above sources were 18 (13 overweight and 5 obese children).

Characteristics of overweight/obese children
The anthropometric details of the 13 overweight children [4 Male] and 5 obese [3 Male] with adequate data were as follows: mean age of 6.4 yrs (SD 0.9), median BMI of 18.2 (range 17.3- 22.7) and a median BMI Z-score of 1.38 (range 1.14-3.10) (Table 5.1). The mean number of days monitored was 4.1 (SD 0.7) and the participants wore the monitors for approximately 13.1 (SD 0.5) waking hours per day. There were 6.2% of total monitoring hours which showed missing data (where the monitor was removed).

Sitting behaviour variables in overweight/obese children
The mean (SD) percentage of waking time spent sitting was 52.4% (5.2), representing 6.9 hrs (SD 0.8). The total number of sitting bouts was 284 (66). The median (range) of 50% and 90% of sitting bouts length were ≤50.0s (40.0-50.0) and ≤3.5 min (3.0-6.0), respectively. The mean (SD) Fragmentation Index was 41.5 (9.6) (Table 5.2).

5.5.1.2 Healthy weight children

Forty seven children from 3 schools participated in the study, 26 of which were of healthy weight (14M: 12F) (Figure 5.1). Their mean age was 6.4 yrs (SD 0.9), median BMI 15.8 (range 12.3-16.9) and median BMI Z-score 0.04 (range - 3.24-0.66). The mean (SD) number of days monitored was 4.0 (0.8), as the participants wore the monitors for a mean (SD) of 13.0 (0.5) waking hours per day. There were 8.2% of total monitoring hours which showed missing data (where the monitor was removed) (Table 5.1).

Sitting behaviour variables in 26 healthy weight children

During the waking hours, the mean (SD) percentage of waking time spent sitting was 53.0% (6.4) representing 6.8 hrs (0.9). The mean (SD) total number of sitting bouts was 280 (65). The median (range) of 50% and 90% of sitting bouts were ≤50.0s (40.0-50.0) and ≤3.5 min (2.0-6.0), respectively. The mean (SD) Fragmentation Index was 42.1 (12.7) (Table 5.2)

5.5.1.3 Overweight/Obese children vs. healthy weight children

Descriptive participant characteristics and sitting behaviour of the 18 overweight/obese (13 overweight and 5 obese children) and 26 healthy weight children are shown in Table 5.1. The groups did not differ significantly in age. As expected, weight, BMI, and BMI Z-score were significantly higher in the obese group compared to the non-obese group.

Table 5.1 Characteristics of overweight/obese children vs. healthy weight children

Variable	18 (13 Overweight, 5 Obese,)	26 healthy weight	P-value
Age (years)	6.4 (0.9)	6.4 (0.9)	0.87
Weight (kg)	26.5 (4.3)	20.5 (2.6)	0.00
Weight Z score	1.26 (0.81)	-0.46(0.90)	0.00
Height (cm)	117.9 (7.8)	114.8 (6.2)	0.14
Height Z score	-0.03 (1.19)	-0.63 (0.88)	0.09
BMI (kg/m2)#	18.1 (17.3-22.7)#	15.8 (12.3-16.9)#	0.00
BMI Z score#	1.37 (1.14-3.10)#	0.04 (-3.24-0.66)#	0.00

Normally distributed data is presented as mean (SD). BMI and BMI Z-score were not normally distributed so presented as median (range) #.

Sitting behaviour variables of overweight/obese children vs. healthy weight children

The mean total sitting time during waking hours was 6.9 hrs (SD 0.8) in the obese and overweight group, and 6.8 hrs (SD 0.9) in the control group, which were similar, with no significant difference between groups (2-sample t-test p = 0.81). The mean (SD) percentage of waking time spent sitting, the total number of sitting bouts, the median of 50% and 90% sitting bouts length, and the mean (SD) Fragmentation Index were also not significantly different between the groups (Table 5.2).

Table 5.2 Sitting behaviour characteristics of overweight/obese children vs. healthy weight children

Variable	18 (13 Overweight,5 Obese)	26 healthy weight	P-value
Total sitting time (hr)	6.9 (0.8)	6.8 (0.9)	0.81
% Sitting time	52.4 (5.2)	53.0 (6.4)	0.73
Number of sitting bouts	284 (66.0)	280 (65)	0.87
50% sitting bout length(s)#	50.0s (40-50)#	50.0s (40-50)#	0.71
90% sitting bout length(min)#	3.5(3.0-6.0)#	3.5(2.0-6.0)#	0.97
Fragmentation index	41.5 (9.6)	42.1 (12.7)	0.86

Normally distributed data is presented as mean, except for 50% and 90% sitting bout length; they were not normally distributed so presented as median (range) #.

5.5.1.4 Overweight, Obese children and healthy weight children

We analysed sitting behaviour in three groups (overweight, versus obese, versus healthy weight). Descriptive participant characteristics are shown in Table 5.3 There was no significant difference in the age between the three groups. However, BMI and BMI Z-score were significantly different.

Table 5.3 Characteristics of overweight, obese children and healthy weight children

Variable	Overweight (n=13)	Obese (n=5)	Healthy weight (n=26)	P-value*
Age (years)	6.5 (1.)	6.1 (0.7)	6.4 (0.9)	0.68
Weight (kg)	25.4(2.7)	29.6 (6.3)	20.5 (2.6)	0.00
Weight Z score	0.96 (0.53)	2.08 (0.91)	-0.46(0.90)	0.00
Height (cm)	118.3 (6.2)	117.0(11.9)	114.8 (6.2)	0.29
Height Z score	-0.07 (0.99)	0.07 (1.75)	-0.63 (0.88)	0.19
BMI (kg/m2)#	17.9(17.3-18.8)	21.9(20.0-22.70)	15.8 (12.3-16.9)#	0.00
BMI Z score#	1.35(1.69-1.14)	3.03(2.20-3.10)	0.04 (-3.24-0.66)#	0.00

Normally distributed data is presented as mean (SD). BMI and BMI Z-score were not normally distributed so presented as median (range) #. ANOVA*

Sitting behaviour variables of overweight, versus obese, versus healthy weight

There were no significant differences between the three groups in all sitting behaviour components, table 5.4.

Table 5.4 Sitting behaviour characteristics of overweight, obese children and healthy weight children

Variable	Overweight (n=13)	Obese (n=5)	Healthy weight (n=26)	P-value*
Total sitting time (hr)	7.0 (0.7)	6.4 (0.8)	6.8 (0.9)	0.32
% Sitting time	53.7 (5.5)	49.1 (4.6)	53.0 (6.4)	0.33
Number of sitting bouts	297 (65.4)	249 (59.8)	280 (65)	0.37
50% sitting bout length(s)#	#50.0s(40.0-50.0)	50.0s(50.0-50.0)	50.0s (40-50)#	0.27
90% sitting bout length(min)#	# 3.0(3.0- 6.0)	4.0(3.0-5.0)	3.5(2.0-6.0)#	0.93
Fragmentation index	42.5(10.4)	38.8 (7.12)	42.1 (12.7)	0.83

Normally distributed data is presented as mean, except for 50% and 90% sitting bout length; they were not normally distributed so presented as median (range) #. ANOVA*

The correlation between BMI Z score and number of sitting bouts of all participants (n=40).

Figure 5.2 shows no correlation between BMI Z score and number of sitting bouts of all participants (healthy weight, overweight, and obese) , R-Sq = 0.3% ,P-value=0.72.
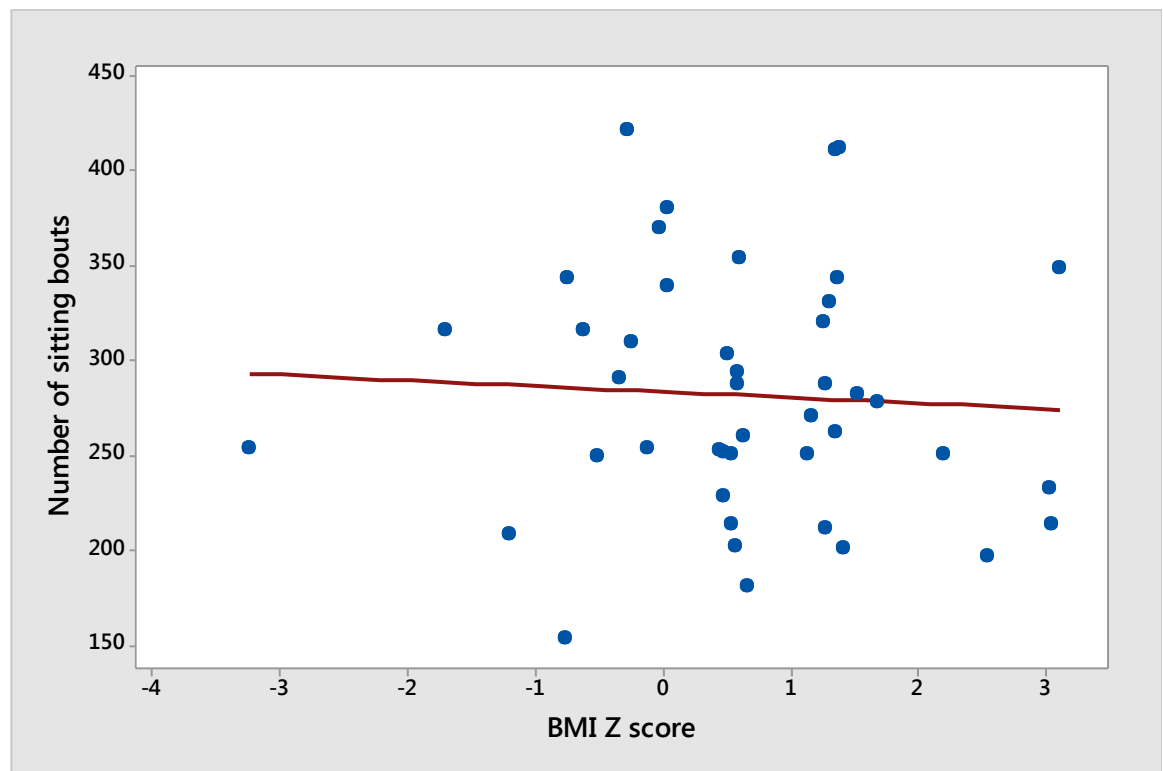
Figure 5.2 Correlation analysis of BMI Z score and number of sitting bouts of all participants **(**healthy weight, overweight and obese)**.** Each participant represented by a dot (n=44).

5.6 Discussion

5.6.1 Main findings and study implications

The purpose of this study was to carry out a pilot study to explore whether there are differences between overweight/obese and non-obese children in their normal free-living sitting behaviours. To measure this behaviour, we used components of sitting behaviour, previously described in Section 2.4.

In the present study, the sitting behaviour components were similar and not significantly different between the overweight/obese children and healthy weight children (Table 5.2). Both groups spent the majority of their waking time sitting. For both groups, 90% of the sitting bouts lasted ≤3.5 min.

There were also no significant differences between the overweight, versus obese, versus healthy weight in all sitting behaviour components. Sitting behaviour characteristics of these three categories are show in table 5.4.

5.6.2 Sample size calculation

The results from this study showed that there was a small difference of the sitting behaviour components between overweight/obese and healthy weight children. This allowed us to estimate the sample size that would be required to detect a sample size of a given magnitude at a pre-specified level of significance (268;269). If there was more than one outcome, then the largest sample size should be chosen so that all the outcome measures are fully powered (270).

From this pilot data, a sample size of 7,275 in each group would be required to detect a difference in the number of sitting bouts with a significance level p = 0.05 and 80% power. This is a very large number of children.

This result suggests that the pilot study data is sufficient to exclude all but a very small difference between the obese/overweight children and controls in measures of sitting behaviour. Such large studies may be difficult to conduct if the difficulties we encountered recruiting sufficient obese and overweight children are replicated. So the power calculation from this pilot study shows

that further studies may need too many participants to find significant differences between the both groups.

5.6.3 Comparisons with other studies

Our results are not directly comparable to any other study results due to the lack in currently available data regarding the differences between overweight/obese and healthy weight children in the components of sitting behaviour using the activPAL™.

A study by Hughes et al. (271) measured habitual physical activity and sedentary behaviour in 53 clinical sample of obese children, mean (SD) age 8.6 yrs (2.0), mean (SD) BMI 27.3 (4.1), and compared with 53 non-obese controls, mean age 8.7 yrs (2.1), mean BMI 16.4 (1.5). These authors found that the total physical activity was significantly lower in obese children, but there was no significant difference in time spent being sedentary between the two groups of children; the % of time spent in sedentary behaviour during waking hours was 80.9% (SD 6.6) per 68.0 hrs (SD 18.1) monitoring time in the obese group, and 79.3% (SD 6.2) per 65.8 hrs (SD 14.8) monitoring time in the non-obese group (271). They used the Actigraph with ≥1,100 cpm used to define the sedentary behaviour; this result was consistent with our study results in that we also showed no significant difference between overweight/obese and non-obese children in their sitting time. However, the time spent sedentary was much greater in Hughes et al.'s study. The difference might be methodological; the Actigraph could misclassify standing time as sedentary time, so the sedentary time may include standing (99;190). Moreover, Lyden et al. (211) observed that the overestimation of total sedentary time will be increased when the sedentary time cut point is increased (211). In Hughes et al.'s (271) study, the ≥1100 cpm was used to estimate the sedentary behaviour, which was much higher than the other cut points which had been used to define the sedentary time, for example 100 and 150 cpm.

Levine et al. (237) developed a physical activity monitoring system involving inclinometers and triaxial accelerometers to record body position and motion over 10 days in 10 (five females and five males) mild obese adults (mean (SD), BMI 33 (2) kg/m²) and 10 (five females and five males) non-obese adults (mean

(SD), BMI 23 (2) kg/m^2). They reported that the obese participants are on average seated 2 hrs longer per day than non-obese participants, and non-obese participants stood for about 2.5 hrs longer per day than obese participants. These sitting/standing behaviour patterns were nearly the same even when obese participants lost weight or lean participants gained weight (237).

Levine's study results (237) are not consistent with our results. These large differences are quite different from the data we recorded in children. It is noteworthy that no other study published has replicated Levine's findings. Thus, it is possible that Levine's results reflect a small and abnormal population of subjects, and the data cannot be generalised to the obese/overweight population as a whole.

The inconsistency between our study results and Levine's study results may also be due to differences in monitoring methodology. Levine used a specially developed physical activity monitoring system validated for measurement of posture transitions in laboratory environments (272), rather than under free-living conditions. However, the activPAL™ is a valid tool for measuring sitting time in free-living environments, and thus allows one to examine children in a more natural setting (102;208).

A final possibility for the difference between the two studies is that the patterns of sitting and standing that characterised Levine's participants are not present in young children. Some studies have observed that sedentary time increases (107;110) and breaks decrease by age (88;107) ; e.g. the sedentary time in a cross-sectional study was 6.07 (0.06) hrs/day in children (n = 811), and 7.48 (0.11) hrs/day in adults (n = 636) (110). In the longitudinal study by Mitchell et al. (107), they observed that the sedentary time was increased from 7.1 (1.1) hrs/day at age 12 (n = 5429) to 8.6 (1.2) hrs/day at age 16 (n = 1971), and the sedentary bouts lasting ≥30 min (less breaks) increased at age 16 (107). In another longitudinal study by Kwon et al. (88), they concluded that from ages 5 (n = 423) to 15 yrs (n = 344) the breaks in sedentary time decreased by >200 times/day (88). All the above studies (88;107) used the Actigraph to measure the sedentary time; it is therefore likely that sedentary behaviour (sitting) patterns are not similar in different age groups.

Also inconsistent with our results, a cross-sectional study by Kreuser et al. (273) compared the sedentary levels in 37 healthy weight children (mean age 8.7 yrs (SD0.9), BMI 16.2 (SD 1.2)) vs. 55 overweight children (mean age 9.3 yrs (SD 1.2), BMI 24.6 (SD 2.9)) using an accelerometer (AiperMotion 440) and a questionnaire (children's parents completed the questionnaire), and observed that overweight children spent significantly (p = 0.05) more time being sedentary (from both subjective and objective methods) than the healthy weight children. The difference between our study and Kreuser et al.'s study might be methodological; to date, the accelerometer, which was used in their study, has not been validated to assess its ability to measure sedentary time. Moreover, there was a significant difference (p = 0.04) in the mean age between the two groups: the overweight children were older, and, as mentioned above, sedentary time increases (107;110) by age, Therefore, the different ages in both groups might affect the results.

5.6.4 Study strengths and limitations

The most important limitation was the relatively limited number of obese participants - they were only 5 obese children in the study. While there was a significant difference in BMI between both groups, the very small number of obese children in the present study is an important limitation. No sample size calculation was made, but the sample was considered sufficiently large to establish feasibility of the study in a clinical setting, and to provide data on which future power calculations could be based. The future power calculations would need to consider what meaningful group differences might be, but at the time of the research there was no evidence on which to identify how large a difference might be to become meaningful clinically or biologically.

Another limitation is the use of the 2s MSUP setting. Our study 2 (chapter 4) showed that 2s was good for pre-schoolers (mean age (SD) 4.1y (0.5) but we used the same setting here in older children (mean age (SD) 6.4 (0.9)). The main reason for not varying the MSUP in the older children in the clinical studies was that, unlike with the pre-schoolers where we had criterion validity data because of the availability of direct observation study (208), for the older children no such criterion validity data were available, so the validity of any MSUP in older children is unclear.

5.6.5 Conclusion

This pilot study of 18 obese and overweight children compared to 26 healthy non-overweight children did not show a significant difference in the sitting time, number, and duration of sitting bouts, and the Fragmentation Index between healthy weight children and overweight/obese children. The participation in sitting behaviour was equally high in both groups, with short sitting bouts. The results of this pilot study should be confirmed by further studies. However, a power calculation from our pilot study shows that a definitive study will require a huge sample size to demonstrate significant differences between overweight/obese and healthy weight children. In this light, it this may be not helpful to carry out further studies.

Chapter 6

Sitting behaviour in Girls vs. Boys

6.1 Introduction

Many studies have found high levels of sedentary behaviour in children and youths (69;110) ; this is true even in preschool children (40;172). Interestingly, previous studies (107;110), using the Actigraph, have observed that girls spend more time in sedentary behaviours than boys. Additionally, some studies demonstrated that boys were more active than girls; this has been noted both in older (26;29;274) and younger children (275). These previous studies have focused largely on the total amount of sedentary behaviour. However, a recent study by Kwon et al. (88), employing the Actigraph, reported that the number of sedentary breaks was also slightly, but significantly, lower in girls than boys (88).

As yet, there has been little research on the possible health consequences of interruptions in sedentary time in children (38;90). In adults, there is some evidence that short bouts of objectively measured sedentary behaviour may reduce negative health consequences compared to prolonged bouts, even if the total amount of sedentary time is the same (47).

The activPAL™ has been validated against direct observation for measurement of both posture allocation and posture transitions in adults (224) and preschool children (102). By using the activPAL™, important features of sitting behaviour, including both the amount or volume and the pattern of sitting time in terms of the number and duration of breaks, can be characterised objectively by a few fundamental metrics. They were described in Section 2.4.

As mentioned above, previous studies (107;110) have observed differences in sedentary behaviour between boys and girls using accelerometers. These studies defined sedentary behaviour as low movement or low energy expenditure (210;211). More recently, sedentary behaviour has been defined by SBRN as the time spent in sitting or lying postures during waking hours. The issue of gender differences has moved onto a focus on sitting (41).

This shift in focus has been facilitated by new opportunities to measure sitting behaviour accurately using a newer generation of devices, such as the activPAL™.

The present study was a preliminary investigation exploring ways in which the activPAL™ might reveal new insights into sitting behaviour. In this study, we aimed to test whether activPAL™ measured sitting times and breaks in sitting differed between boys and girls to investigate possible effects of gender on sitting behaviour

6.2 Methods

6.2.1 Participants

The data used in this study were previously used in study 1b and study 3. The procedures for recruitment were described in the previous chapters (3 and 5). In brief, 23 children from study 1b and 47 children from study 3 (from the three schools, figure 5.1), where they were merged. The data represented a convenience sample of 70 free-living healthy children was recruited from nurseries and schools in Glasgow and Edinburgh, Scotland. Informed consent was obtained from the parents/guardians of all participants.

Sex, age, height, and weight were recorded at the start of the study. BMI was then calculated from the height and weight measures (kg/m2). Anthropometric measures were described in section 2.1.

6.2.2 Measurement of sitting variables - sitting time, breaks in sitting, sitting bouts - using the activPAL™

Sitting time was measured objectively using an activPAL™ monitor (PAL Technologies Ltd., Glasgow, UK). Children were asked to wear the monitor continuously; 24 hrs a day, for seven consecutive days (see Section 2.2 for more details).

6.2.3 Data Processing

The activPAL™ uses algorithms to record time spent sitting/lying, standing, walking, and transitions. The data from all devices were downloaded to a computer for data analysis. Data processing was described in detail in Section 2.3.

The results obtained from Study 2 (Chapter 4) showed that the 2s MSUP activPAL™ is the most appropriate MSUP setting. A 10s MSUP setting is not suitable for this age group. Therefore, the results of this chapter have been calculated with a 2s MSUP. For the purposes of comparison, we also calculated the results with the 10s default setting, and, where appropriate, these will be mentioned briefly in the discussion.

6.3 Statistical analyses

Regression analysis was used to investigate which explanatory variables (age, BMI, sex) were predictive of the outcomes of total sitting time, % sitting time, number of bouts, 50% and 90% sitting bout length, and Fragmentation Index. Two-sample t-tests were used to detect the differences between girls and boys.

6.4 Results

6.4.1 Participant description

Of the 70 children studied, sixty-two children provided adequate data, and they were included in the final analyses. Eight children were excluded, 7 children wore the monitors less than 3 weekdays during the study, and one child lost the device. The participant characteristics of the 32 girls and 30 boys are shown in Table 6.1. There were no sex differences in age, weight, height, BMI, and BMI Z-scores. In all children, seven children (4F, 3M) had a BMI Z-score of +1.64.

Table 6.1 Participant characteristics mean (SD)

Variable	Girls (n = 32)	Boys (n = 30)	P-value
Age (years)	5.7 (1.1)	5.8 (1.4)	0.60
Weight (kg)	21.2 (4.2)	21.4 (3.9)	0.90
Height (cm)	112.1(7.5)	113.5 (7.5)	0.50
BMI (kg/m2)	16.9 (2.1)	16.4 (2.0)	0.40
BMI Z-score	0.67 (1.07)	0.46 1.32	0.48

For all children, the mean (SD) monitoring time was 4.0 days (0.2), of which a mean of 12.6 hrs (0.7) was in waking hours. Missing data (where monitor was removed - see Section 2.3.1) accounted for a mean of 6.9% (SD 1.6) of total monitoring hours.

6.4.2 The sitting behaviour variables during waking hours, in girls vs. boys using 2s MSUP

The percentage of waking time spent sitting was significantly higher in girls, the mean (SD) (54.4 (5.6) % compared to boys (50.9 (5.6), 2-sample t-test, p value <0.02). The total sitting time per waking hours was 6.9 hrs (0.8) vs. 6.5 hrs (0.9), p-value <0.08, in girls vs. boys, respectively. There were no significant differences in the number of sitting bouts, fragmentation index, and in 50% and 90% sitting bout length between girls and boys (Table 6.2).

Table 6.2 Sitting behaviour variables in girls vs. boys, the mean (SD**)**

Variable	2s MSUP		P-value
	Girls (n = 32)	**Boys** (n = 30)	
Total sitting time (hr)	6.9(0.8)	6.5 (0.9)	0.08
% Sitting time (defined as-sit/lie only)	54.4 (5.6)	50.9 (5.6)	0.02
Number of sitting bouts	280 (78)	288 (54)	0.60
50% sitting bout length(s)	45.8(4.9)	45.0 (5.1)	0.54
90% sitting bout length(min)	3.4 (1.5)	3.2 (1.0)	0.14
Fragmentation Index*	41.4(13.5)	45.4(10.0)	0.18

*The Fragmentation Index = the number of sitting bouts/total sitting time measured in hours

A univariate analysis was undertaken first to see if there was any relationship between sitting behaviour metrics (total sitting time, % sitting time, number of bouts, 50% and 90% sitting bout length, and Fragmentation Index) and the explanatory variable (predictor, age, BMI, sex). Neither age nor BMI were significant. Only sex was a predictor independent of age and BMI.

6.5 Discussion

6.5.1 Main findings and study implications

This pilot study investigated whether or not differences between boys and girls in sitting behaviour variables could be identified using activPAL™ measurements of sitting behaviour in free-living young children. To our knowledge, no previous study has examined the ability of the activPAL™ to detect sex differences in sitting behaviour and breaks in sitting for a young age group. Therefore, this study carried out a preliminary investigation to see if previously reported between-sex differences in sedentary behaviour as measured by more traditional accelerometers were replicated using an accelerometer designed to measure sitting and breaks in sitting, and to provide quantitative data needed so that future studies could be planned and powered properly.

The percentage of waking time spent sitting was significantly lower in boys compared to girls, amounting to a mean difference of around 0.4 hrs per day. The number of sitting bouts, reflecting the number of breaks in sitting, and Fragmentation Index were slightly higher in boys than girls, but they did not differ significantly. No significant gender difference was found in the 50% and 90% sitting bout length (Table 6.2). The absence of differences may possibly be due to the sample size was relatively small.

By using a 10s MSUP, the percentage of waking time spent sitting was significantly lower in boys compared to girls, 50.8 (6.1) vs. 54.5 (5.7), 2-sample t-test, p-value <0.02. No significant gender difference was found in other sitting behaviour variables (Table 6.2). The results of 10s looked similar to the results of 2s MSUP findings in the gender differences.

This extends the field beyond the well-known observation of higher levels of sedentary behaviour in girls than boys in the previous studies (88;107;110). These studies, described briefly below, mainly used the Actigraph to measure the sedentary time.

The present study suggesting gender differences in sitting time but not other characteristics illustrates how the use of the activPAL™ may reveal insights not readily available from the previous studies using earlier monitors, such as the Actigraph, that has been, until recently, used to quantify SB time as time spent in low intensities of activity but not sitting time per se. The activPAL™ is a valid device for measuring total sitting time and breaks in sitting. Thus, the activPAL™ should be considered for use in studies designed to assess SB (sitting or reclining) (210;211;276).

6.5.2 Comparisons with other studies

Our study was the first to investigate the differences between boys and girls in sitting behaviour variables using the activPAL™ in free-living young children. In previous studies (107;110), sedentary time, defined not as sitting but as a lack of activity, is known to be different between girls and boys, but there is no evidence between sex differences in either total sitting time or other sitting variables using activPAL™, especially at the young age of the sample we were dealing with.

A recent longitudinal study by Kwon et al. (88) described changes in the frequency of breaks in sedentary behaviour from ages 5 to 15 using a pragmatic definition of <100 Actigraph cpm to define a sedentary behaviour, and break if accelerometer counts were ≥100 cpm (88). In this study, at age 5 yrs [201M: 222F], the mean (SD) daily time spent in sedentary behaviour was 3.6 hrs (0.8) in boys and 3.7 (0.8) in girls, the mean (SD) number of sedentary breaks during waking hours was 525 (73) in boys and 519 (75) in girls, the sedentary time was slightly higher in girls than boys, but the frequency of sedentary breaks was slightly but significantly higher in boys than girls (within 2 times/hr, P <0.01) at ages 11, 13, and 15 (88) . These results were similar to our results: girls were more sedentary and had less breaks than boys. However, our study shows much higher levels of sitting time (mean (SD), 6.5 hr (0.9) in boys and 6.9 hr (0.8)) in

girls, and a much lower number of breaks in sitting behaviour, mean 288 (54) in boys and 280 (78) in girls, during waking hours, compared to the data reported by Kwon et al. (88) at age 5. Differences between the two studies might reflect a real biological difference between the samples. However, it is more likely that the differences are methodological, since the Actigraph is not designed to accurately distinguish between sitting and standing (276). A recent validation study in adults (210;211) of the Actigraph and activPAL™ against direct observation for measurement of both sedentary time and breaks in sedentary time, concluded that the Actigraph underestimated the sedentary time, especially with <100 cpm cut point (99), and overestimated the breaks in sedentary time (211). However, the activPAL™ provides a precise estimate of both total sitting time (sitting or lying) and break in sitting (210;211). So, at present, we should have less confidence in gender differences in sitting time and breaks in sitting time as measured by the Actigraph.

Similar to our results, other studies also observed that girls spent more time in sedentary behaviour than boys. For example, Matthews et al. (110) and Mitchell et al. (107), both using the ActiGraph, found that the mean time spent in sedentary behaviour for girls was significantly higher than boys in older children and adolescents. In Matthews et al.'s study in the United States [386M: 425F] the mean times spent in sedentary behaviour, using the Actigraph and defined as <100cpm, were: in boys: 41.4 %, 6.0 hr/day; in girls: 43.4 %, 6.1 hr/day, t -test, p <0.002 at age 6-11 yrs. Generally, they observed that females were more sedentary than males (110). In the Mitchell et al. study in the UK [The Avon Longitudinal Study of Parents and Children (ALSPAC)], at age 12 yrs [2,591M: 2,845F], the mean times spent in sedentary behaviour, using the Actigraph and defined as <100cpm, were 7.3 hr (1.1)/day in girls, and 7.0 hr (1.1)/day in boys (107).

6.5.3 Study strengths and limitations

For total sitting time, the p-value was 0.08, which is close to being significant. The lack of statistical significance could be related to the sample size not being large enough to detect the gender differences. From our pilot study data, a sample size of 81 children in each group would be required to detect the difference in the total sitting time with a significance level p = 0.05 and 80%

power. The number of children is similar for the number of bouts, 50% and 90% sitting bout length and Fragmentation Index. Hence, the present observations need to be confirmed in a larger and more representative sample. From our data, a sample size of 1,283 children in each group would be required to detect the difference in these sitting behaviour variables with a significance level $p = 0.05$ and 80% power.

In this study the weekends were excluded to avoid any confounding issues arising from weekend/weekday differences. However, it is likely that this reduced our ability to detect gender differences in sitting behaviour variables, as differences between sexes during weekdays might be less obvious because behaviours might be more similar/constrained during weekdays than compared to the weekend.

6.5.4 Conclusion

This study suggests that girls, mean (SD) age 5.7 (1.1), may spend more time in sitting than boys, not just more time 'sedentary'. The gender differences in sitting time using the activPAL™ in free-living young children can be identified by a relatively small study, suggesting the potential value of the activPAL™ in studies of between-group differences in sitting behaviour. Previous studies had established that sedentary behaviour is, on average, higher in girls than boys. However, no gender differences on the number or period of sitting bouts were observed. The present study suggests that the activPAL™ seems able to identify a sex difference in sitting time in young children, and this may be helpful as a proof of concept for a future use of activPAL™ to detect the differences between other groups.

Chapter 7

General discussion

7.1 General discussion and conclusions

Sedentary behaviour (SB) has an adverse effect on health, independent from overall physical activity levels. Several studies have demonstrated that SB (mainly TV viewing) is associated with a range of poor health outcomes, such as obesity, hypertension, and other cardio-metabolic risk factors in adults and children (38;39;277). However the role of objectively measured sedentary time in influencing health in children and adolescents is currently unclear and is being debated.

A difference in sitting and standing between obese and non-obese adults was highlighted by Levine et al. (237) , who observed that obese individuals sit longer per day than lean individuals. This finding has yet to be replicated in children and in other adults, and is evidence from a single study only.

Emerging evidence, using the ActiGraph, suggested that, not only the total sedentary time, but the pattern of sedentary time may be independently associated with health outcomes in adults. Interruptions in objectively measured sedentary time (breaks) were associated with markers of cardiometabolic risk (WC, BMI, 2-h plasma glucose, and C-reactive protein levels) (42). Studies investigating the relationship between breaks in SB and health outcomes in children are still limited, or do not exist in children of a young age. Recently, Saunders et al. (90) investigated the association between breaks in SB and cardiometabolic risk in children aged 8–11 yrs, with a family history of obesity. Using the ActiGraph, the results suggested that breaks in sedentary time are beneficially associated with markers of cardiometabolic risk (90;118). But, the ActiGraph is not a valid tool for measuring breaks in SB (211).

Due to the increasing interest in breaks in SB and the obvious interest in examining levels of SB, a device that is both a valid and reliable measure of SB components would be an important research tool.

While the ActiGraph and the Actical, for example, are used to measure SB and breaks in SB, their measurements are dependent on thresholds. It has been suggested that the use of such thresholds to determine sedentary time could lead to inaccuracy, as these types of

accelerometer rely on measurements of the lack of movement rather than directly measuring body position, and this analysis may include other activities, such as standing (190;211). According to the Sedentary Behaviour Research Network definition, SB is activity with low energy expenditure during waking while in a sitting or lying position (41). Activities such as standing, which would previously have been considered sedentary, should now be considered as different activity behaviours.

Furthermore, the results which had been obtained from some validation studies in assessing the ability of the ActiGraph to measure breaks in SB showed that the monitor is not a valid measurement tool for assessing breaks in SB in adults (211), but, until now, no study has examined the ability of the ActiGraph monitor to measure breaks in SB in children.

The use of inclinometer-based activity monitors, such as the activPAL™, has allowed researchers to directly measure periods of sitting/lying, and standing and stepping, and there use has been encouraged for studies examining sitting behaviours in detail.

The activPAL™ is a valid monitor for measuring sitting time (ST) in different age groups (101;102;224) . Moreover, the activPAL™, using a 10s MSUP, has been shown to accurately detect breaks in ST in adults and older children, 9-10 yrs old, but not in young children. The validation studies which had been (101;224) conducted in young children, using a 1s MSUP, observed that the activPAL™ overestimated the number of breaks in ST compared with direct observation data (102;103). The negative results which had been obtained from these studies (102;103) might be because a 1s MSUP was not suitable for this young age group. However, the effect of changes in MSUP and the optimum activPAL™ setting of MSUP to define a change in posture for measurement of sitting time and breaks in sitting time are not known.

Four different studies have been conducted in this thesis. The first studies, Study 1a and Study 1b (Chapter 3), were conducted in the laboratory and on data collected previously from young children in a free-living environment. Together, these studies aimed to investigate the effect of the change in MSUP activPAL™ setting on ST components, and to

examine if the measures used in adults to characterise their sitting behaviour are also suitable to use in young children.

The results from Study 1 showed that the change in the MSUP activPAL™ setting had a significant effect on breaks in ST, but no significant effect on the total ST. The ST in children can be characterised into total ST, breaks in ST, and the pattern of an accumulation of ST represented by accumulation curves and a Fragmentation Index.

Although the first study reported that the change in the MSUP activPAL™ setting had a significant effect on the breaks in ST, the most suitable MSUP for this age group is still unknown. Therefore, the second study (Chapter 4) was planned to determine the most appropriate MSUP activPAL™ setting for young children. The data was collected from preschool children who wore the activPAL™ and were videoed for 1 hr (direct observation) in the nursery. The activPAL™ data, using the varying settings, was compared with direct observation data. For preschoolers, 2s appeared to be an appropriate MSUP to define breaks in sitting using the activPAL™. No previous studies have examined the most appropriate MSUP activPAL™ setting for different age groups.

From the results of the first and second studies, the sitting behaviour in young children can be characterised by simple measures, and a 2s MSUP is the most suitable activPAL™ setting for this age group. So, the practical aspects of measurement and describing the sitting behaviour components were standard.

Until now, no previous studies have examined the effect of the activPAL™ MSUP on ST components. The results from Study 1b suggest that children's sitting behaviour may be different from adults' behaviour; compared with previous studies in adults (96;100) , children tend to break their ST more frequently (more breaks), with shorter sitting bouts than adults. However, our sample was not a representative sample, and the aim of Study 1b was not to investigate the differences between children and adults in sitting behaviour.

The third study, a pilot study (Chapter 5), was conducted to compare the sitting behaviour components between overweight/obese and healthy weight children, while the fourth

study (Chapter 6) aimed to investigate the difference in these components between boys and girls.

In the third study, both groups spent the majority of their waking time sitting, with the sitting bouts periods lasing ≤3.5 min, but there was no significant difference in the sitting behaviour components between the two groups. The lack of positive results may be explained by the fact that the sample size of obese children might not have been large enough. Our results were similar to other studies' results using the ActiGraph, in that there was no significant difference between overweight/obese and healthy weight children in their SB (271). They are, however, inconsistent with the previously published study which reported that overweight children had significantly higher SB than healthy weight children (273). However, it is difficult to compare the results of our study with previous reports, as no previous study has investigated the difference in objectively measured ST between these two groups.

In the fourth study, the percentage of waking time spent sitting was significantly higher (p = 0.02) in girls compared to boys; however, no gender differences in the breaks in ST were observed, perhaps because the sample size in this study was relatively small. Gender difference in sedentary time have been observed in previous studies using the ActiGraph (107;110), where girls spent more time sedentary than boys. However, these studies did not measure the ST directly, but measured sedentary time as a lack of activity.

7.2 Future Direction

Further studies are needed to determine the most appropriate MSUP activPAL™ setting to define a new posture for different age groups, and the determination of it would also be helpful for future use of the activPAL™. The difference in the sitting behaviour between obese/overweight children vs. non-overweight children may be detected with additional studies on a larger number of obese participants, but the power calculation from Study 3 demonstrates that this further study would require a huge sample size to detect significant differences between the two groups. As a consequence, thus study may not be of sufficient interest to do an additional study.

The power calculation from Study 4 shows that a sample size of 1,283 children in each group would be needed to find the gender difference in the sitting behaviour variables with a significance level p = 0.05 and 80% power. Therefore, the gender difference in these variables may be needed to confirm with future cross-sectional studies using the activPAL™ with a larger and more representative sample.

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Appendices

Appendix A

Study information sheet for the parent

School of Medicine
Section of Child Health/Human Nutrition

20 April 2012

Dear Parent/guardian,

We are part of a team of researchers based at the University of Glasgow looking at differences in the time spent sitting and standing between overweight and more normal weight children. We are interested in this because there is evidence that adults who are overweight spend less time standing than more normal weight adults. At present, we have no evidence whether this is also true in children.

We can measure whether children are standing or sitting by using a small activity monitor, about the size of a matchbox but a third as thick. This monitor can detect whether a young child is sitting or standing.

We would like to invite your child to take part in our study. The study does not require any additional appointments or visits other than their normal attendance at nursery/school

I have enclosed an information leaflet and consent form. If you are interested in taking part in the study after reading the information leaflet, please complete the consent form and return it in enclosed stamped addressed envelope. We will contact you and arrange a meeting to discuss the study in detail.

Yours sincerely

Zubaida Alghaeed,

MD Student

James Paton

Reader in Paediatric Respiratory Medicine

University of Glasgow

School of Medicine
Section of Child Health/Human Nutrition

Pilot study to investigate the differences in breaks and bouts of sedentary time between overweight and normal weight children.

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

Who is conducting the research?

The research is being carried out by Dr James Paton/University of Glasgow, Prof. John Reilly/University of Strathclyde and Dr Zubaida Alghaeed/ University of Glasgow

What is the purpose of the study?

We now know that prolonged sitting and lack of physical activity is a risk factor for some diseases in children such as obesity. In adults, there was a difference between those who were obese and those who were normal weight in time spent sitting and standing during the day. Obese adults had longer periods sitting. However, we do not yet know if there is difference in sitting period between obese and overweight and normal weight children. Sitting for a long time without a break may be worse for health than sitting with a lot of breaks so this may help to explain why some children become overweight or obese.

Sitting time will be measured by small lightweight monitor held in place on the thigh with an adhesive dressing.

Why has my child been invited?

This is a small study. We are inviting all overweight children who previously attend Dr. Shaikh's weight management clinic at Yorkhill hospital, children who are attending the Active Children Eating Smart programme run by NHS Greater Glasgow and children in the primary schools who are obese or overweight to take part. We also will be inviting children with normal body weight in different schools to compare their time sitting with the children from the weight management clinic. We plan to study up to 40 children altogether.

Does my child have to take part?

It is up to you and your child to decide. We will describe the study and go through this information sheet, which we will then give to you. You will be asked to sign a consent form to show that your child have to take part. You and your child are free to withdraw at any time, without giving reason. This would not affect the standard of care you receive or your future treatment.

What does taking part involve?

The study involves finding out if there is difference in sitting time between overweight and non-overweight children. A small lightweight activity monitor (the activPAL) will be worn on the mid thigh. The researcher will first attach the monitor to your child at clinic or school. We would then like them to wear this small monitor all the time for 7 days. At the end of the 7 days, the study ends and we will collect the monitor from the children at clinic or at school. We will also measure your child's height and weight at clinic or at school. We will ask you to write when you putting the monitor on and taking it off your child during 7days. The monitor is not waterproof, so you will have to remove and reattach the monitor for showering, bathing or swimming.

What happens to the information?
Your identity and personal information will be completely confidential and known only to the researcher. The information obtained will remain confidential and stored within a locked filing

cabinet. The data are held in accordance with the Data Protection Act, which means that we keep it safely and cannot reveal it to other people, without your permission.

What are the possible benefits of my child taking part?

It is hoped that by taking part in this research you child will be providing valuable information regarding the differences in the sitting period between obese and non obese children and this will be helpful in future studies of the prevention and treatment of obesity in children

Who has reviewed the study?

This study has been reviewed by the NHS Greater Glasgow and Clyde Research Ethics Committee.

If you have any further questions?

We will give you a copy of the information sheet and signed consent form to keep. If you would like more information about the study and wish to speak to someone **not** closely linked to the study, please contact Dr. Guftar Shaikh

Contacts:

Dr James Paton

E-mail: james.paton@glasgow.ac.uk

Tel: +44 (0)141 201 0237

Prof. John Reilly

E-mail: john.j.reilly@strath.ac.uk

Tel: 0141-950-3152

Dr Zubaida Alghaeed

E-mail: z.alghaeed.1@research.gla.ac.uk

Tel: 07748597174

Dr. Guftar Shaikh

E-mail: guftar.shaikh@nhs.net

If you have a complaint about any aspect of the study?

If you are unhappy about any aspect of the study and wish to make a complaint, please contact the researcher in the first instance but the normal NHS complaint mechanisms is also available to you.

Thank-you for your time and co-operation

Appendix B

Study information sheet for the child

Child information sheet
(to be read to child)

We would like you to help us with our project.

This will involve you wearing 1 small box; this is the picture of it (like a tiny phone) on your leg. <u>If you wear this box you will help us to do our study.</u>

When you are wearing the box on you can keep doing what you normally do at nursery/school and home such as playing, drawing or painting.

If you don't want to wear it or want to take them off once we have started that is ok – you just need to tell me or tell your Mum or Dad or Gran or tell your teacher.

Thank you

Appendix C

Parent consent form

CONSENT FORM

Title of study: **Pilot study to investigate the differences in breaks and bouts of sedentary time between overweight and normal weight children.**

Researcher:

To be completed by parent/guardian

Child's name _____ Date of Birth _____

Address _____

Post Code _____Tel No _____

Parent's Work Tel No _____or_____

Emergency Contact Name _____ Tel no._____

Please initial box

1. I confirm that I have read and understand the information sheet dated Dec 11 (version 1) for the above study and have had the opportunity to ask questions.

2. I understand that my child's participation is voluntary and they are free to withdraw at any time, without giving any reason, without my/their legal rights being affected.

3. I agree for my child to take part in the above study.

Name of parent/guardian   Date   Signature

Name of Person taking consent   Date   Signature
(if different from researcher)

Researcher   Date   Signature

1 for parent,   1 for researcher **[Dec 2011 version 1]**
Please return completed forms to weight management clinic staff or head of nursery/school.

Appendix D

ActivPAL activity log

ActivPAL-activity Dairy

Date: **Time:**

Name: **Device Number:**

Date& time when remove/not worn

Date	Time

Return...

Appendix E

Results letter to the parent

Dear Parent/Guardian

We would like to thank you and child's name for helping with our recent research study, which aims to investigate "*The differences in breaks and bouts of sedentary time between overweight and normal weight children.*"

For medical research to progress healthy volunteers like child's name are vital. So we are extremely grateful for your support. This study also formed part of Dr Zubaida Alghaeed's MD project and she is most grateful for your help.

child's name **results:**

Weight: xx kg   Height: xx cm

Body mass index (BMI): xx

During waking hours (defined from first morning transition until 2100):

1. total sitting time – xx% of waking hours
2. sit to stand transitions per hour (averaged over monitoring days) - xx/hr

We have only limited data on healthy children at present, but the average results from children of a similar age group were:

1. total sitting time - xx% of waking hours
2. average sits to stand transition per hour - xx/hr

At present, we can only say that child's name results are similar to the average data from children of a similar age.

The graph below shows your child's sit to stand transitions (averaged over the monitoring days). The pattern of transitions with a small fall after lunchtime and a rise in the late afternoon is also similar to what we have found in other children.
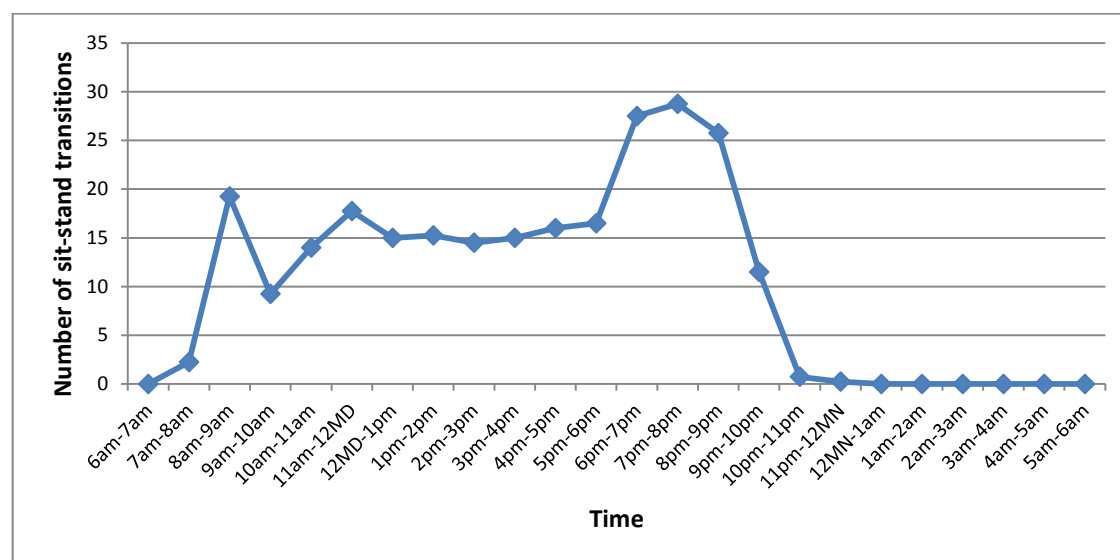
Our findings show that the sitting time and the sit to stand transitions were similar in overweight/obese and non-obese children, but girls were much more sedentary than boys. We are expecting eventually to publish our results in a Scientific Journal (your child's personal information will of course be kept strictly confidential). We hope the results will provide valuable information about the differences in the sit to stand transitions between both groups and this will be helpful in future studies of the prevention and treatment of obesity in children. If you would like any additional information or a more detailed breakdown of your child's results please let us know. Thank you once again for your support.

Kind Regards,

James Paton   Zubaida Alghaeed

Appendix F

Tables

Details of the participants

Table 1 Details of the 18 (13 overweight and 5 Obese) children

Child	Place	Gender	Age [years]	Weight [kg]	Weight Z-score	Height [cm]	Height Z-score	BMI [kg/m²]	BMI Z-score
1A	1	M	5.0	23.8	1.89	117.2	1.68	17.3	1.26
2A	2	F	6.2	25.0	1.15	117.5	0.20	18.1	1.38
3A	2	F	5.3	19.0	0.02	103.0	-1.73	17.8	1.35
4A	2	F	6.9	26.2	0.90	121.0	0.07	17.9	1.14
5A	2	F	6.8	27.1	1.15	123.0	0.58	17.9	1.16
6A*	2	M	6.4	31.5	2.51	120.0	0.34	21.9	3.05*
7A	2	F	6.1	25.1	1.23	115.5	-0.09	18.8	1.69
8A*	2	F	5.1	24.0	1.79	104.0	-1.23	22.2	3.10*
9A	2	M	7.8	28.5	0.84	125.5	-0.22	18.1	1.30
10A	2	M	7.5	28.0	0.95	125.0	0.02	17.9	1.28
11A	3	F	6.4	24.0	0.75	114.8	-0.58	18.2	1.38
12A	3	M	6.5	25.0	0.97	119.0	0.01	17.7	1.36
13A*	3	F	6.3	33.7	2.73	129.0	2.40	20.3	2.20*
14A	3	M	8.1	25.7	-0.05	117.0	-2.07	18.8	1.53
15A	3	F	5.1	23.0	1.50	113.5	0.86	17.9	1.42
16A*	3	M	5.8	22.0	0.61	105.0	-2.03	20.0	2.55*
17A*	4	M	7.1	36.6	2.74	127.0	0.87	22.7	3.03*
18A	4	F	7.4	29.4	1.17	126.0	0.43	18.5	1.28
Mean		10F:8 M	6.4	26.5	1.27	117.9	-0.03	18.2#	1.38#
SD			0.9	4.3	0.81	7.8	1.19	(17.3-22.7)#	(1.14-3.10)#

*Obese-BMI Z score of +1.64.

All data were normally distributed - presented as mean (SD), except BMI and BMI Z-score; they were not normally distributed so presented as median (range) #.

The names of the places are 1- Jordanhill School, 2- Glasgow Libyan School, 3- Edinburgh Libyan School, 4- Active Children Eating Smart programme.

Table 2 Sitting behaviour characteristics in 18((13 overweight and 5 Obese) during waking hours

Child	Total sitting time (hr)	% Sitting time	Number of sitting bouts	50% sitting bout length (s)	90% sitting bout length (min)	Fragmentat-ion Index
1A	7.0	54.1	320	50.0	3.0	45.5
2A	7.4	56.0	412	40.0	3.0	55.7
3A	7.7	55.3	411	50.0	3.0	53.7
4A	7.9	59.6	251	50.0	3.0	31.8
5A	7.1	55.7	270	50.0	4.0	38.2
6A*	5.8	44.5	214	50.0	4.0	37.1
7A	7.8	54.8	278	50.0	5.0	35.8
8A*	7.0	51.8	349	50.0	3.0	50.1
9A	7.1	52.2	331	40.0	3.0	46.6
10A	8.0	63.6	211	50.0	5.0	26.5
11A	5.6	45.7	343	40.0	3.0	60.9
12A	6.6	51.3	262	40.0	3.0	39.5
13A*	6.2	48.6	251	50.0	3.5	40.5
14A	6.3	47.0	282	40.0	3.5	44.4
15A	7.0	54.5	201	50.0	6.0	28.8
16A*	5.6	45.3	196	50.0	4.2	35.0
17A*	7.4	55.5	233	50.0	5.0	31.4
18A	6.4	47.9	288	50.0	4.0	44.7
Mean	6.9	52.4	284	50.0#	3.5#	41.5
SD	0.8	5.2	66	(40.0-50.0)#	(3.0-6.0)#	9.6

*Obese-BMI Z score of +1.64.

Normally distributed data is presented as mean (SD). 50% and 90% sitting bout length were not normally distributed and so are presented as median (range) #.

Table 3 Details of the 26 healthy weight children

Child	Place	Gender	Age [years]	Weight [kg]	Weight Z-score	Height [cm]	Height Z-score	BMI [kg/m²]	BMI Z -core
1B	1	F	5.3	20.0	0.39	111.0	0.01	16.2	0.48
2B	1	M	5.5	17.0	-1.25	111.4	-0.31	13.7	-1.70
3B	1	F	5.5	21.6	0.76	115.3	0.66	16.2	0.47
4B	1	F	5.9	18.2	-0.79	109.0	-1.19	15.3	-0.12
5B	2	M	5.2	19.0	-0.03	107.8	-0.69	16.3	0.59
6B	2	F	5.3	18.3	-0.26	106.0	-1.07	16.3	0.52
7B	2	F	6.6	22.0	0.03	122.0	0.62	14.8	-0.50
8B	2	M	5.6	20.0	0.04	111.5	-0.42	16.1	0.45
9B	2	F	6.2	18.7	-0.82	113.0	-0.72	14.6	-0.62
10B	2	F	7.0	18.0	-1.76	111.5	-1.90	14.5	-0.76
11B	2	M	7.0	18.0	-2.04	113.0	-1.73	14.1	-1.20
12B	2	M	5.5	22.5	1.03	117.5	0.99	16.3	0.60
13B	2	M	6.1	23.2	0.76	119.0	0.50	16.2	0.51
14B	2	M	5.2	18.3	-0.34	106.0	-1.08	16.3	0.59
15B	2	M	5.8	17.6	-0.28	103.4	-1.07	16.4	0.64
16B	2	F	7.2	19.7	-1.24	111.5	-2.10	15.8	0.04
17B	2	F	7.2	21.6	-0.58	114.0	-1.62	15.8	0.04
18B	2	M	7.9	22.4	-0.92	123.5	-0.69	14.7	-0.74
19B	3	F	7.0	22.0	-0.29	114.0	-1.41	16.9	0.66
20B	3	M	6.7	19.9	-0.91	114.5	-1.11	15.2	-0.25
21B	3	M	7.0	21.0	-0.72	117.5	-0.85	15.2	-0.27
22B	3	M	6.9	16.8	-2.60	116.4	-0.97	12.3	-3.24
23B	3	M	8.2	24.5	-0.46	126.5	-0.45	15.3	-0.33
24B	3	F	6.3	20.8	-0.13	116.0	-0.23	15.5	-0.02
25B	3	M	6.9	23.8	0.31	120.5	-0.16	16.4	0.57
26B	3	F	7.1	27.4	0.99	128.0	1.17	16.7	0.54
Mean			6.4	20.5	-0.46	114.8	-0.63	15.8#	0.04#
SD			0.9	2.6	0.90	6.2	0.88	(12.3-16.9)#	(-3.24-0.66)#

Normally distributed data is presented as mean (SD), except BMI and BMI Z-score; they were not normally distributed so presented as median (range) #.

The names of the places are; 1- Jordanhill School, 2- Glasgow Libyan School, 3- Edinburgh Libyan School, 4- Active Children Eating Smart programme.

Table 4 Sitting behaviour characteristics in 26 healthy weight children during waking hours.

Child	Total sitting time (hr)	% Sitting time (defined as-sit/lie only)	Number of sitting bouts	50% sitting bout length (s)	90% sitting bout length (min)	Fragmentation index
1B	7.6	55.9	252	50.0	5.0	33.2
2B	5.5	41.9	316	40.0	2.0	57.1
3B	6.3	50.2	228	50.0	6.0	36.1
4B	7.6	56.7	254	50.0	4.5	33.4
5B	5.9	48.3	294	40.0	3.0	49.8
6B	8.4	68.3	214	50.0	6.0	25.4
7B	8.2	64.0	249	50.0	5.0	30.3
8B	7.3	57.5	251	50.0	4.0	34.7
9B	6.4	52.3	316	40.0	3.0	49.3
10B	6.7	49.1	154	50.0	6.0	23.1
11B	5.6	46.6	208	50.0	6.0	37.5
12B	6.4	47.9	354	40.0	2.0	55.6
13B	8.3	61.5	304	50.0	3.5	37.9
14B	7.6	58.5	288	50.0	3.5	37.9
15B	6.8	53.0	260	40.0	3.0	40.2
16B	5.2	47.8	339	40.0	2.0	65.4
17B	5.5	41.6	380	40.0	2.0	69.0
18B	6.9	52.8	344	50.0	3.0	49.9
19B	6.6	51.7	180	50.0	6.0	27.3
20B	7.5	57.0	310	50.0	3.5	41.1
21B	6.9	54.4	421	40.0	2.0	61.1
22B	7.3	58.1	254	40.0	3.0	35.6
23B	8.0	55.5	291	50.0	4.5	36.5
24B	6.3	46.9	369	50.0	3.0	58.5
25B	6.5	47.7	202	50.0	5.0	31.0
26B	6.9	53.1	251	50.0	4.0	36.4
Mean	6.8	53.0	280	50.0#	3.5#	42.0
SD	0.9	6.4	65	(40.0-50.0)#	(2.0-6.0)#	12.7

Normally distributed data is presented as mean (SD), except 50% and 90% sitting bout length; they were not normally distributed so presented as median (range) #.

Appendix G

Ethical Approval

WoSRES
West of Scotland Research Ethics Service

West of Scotland REC 5
Ground Floor - Tennent Building
Western Infirmary
38 Church Street
Glasgow
G11 6NT

Dr James Paton
Reader in Paediatric Respiratory Medicine
University of Glasgow
Royal Hospital for Sick Children
Dalnair Street, Yorkhill
Glasgow
G3 8SJ

Date   09 March 2012

Direct line   0141 211 2102
Fax   0141 211 1847
E-mail   sharon.macgregor@ggc.scot.nhs.uk

Dear Dr Paton

Study title: Pilot study to investigate the differences in breaks and bouts of sedentary time between obese and non-obese children.

REC reference: 12/WS/0015

Thank you for your letter of 23 February 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC. A list of the sub-committee members is attached.

To clarify point 2 of our 31st January 2012 REC letter, the Statistician on the REC had a concern about how you are planning to select the controls, but not simply about demographics. He was concerned that you will ask the teachers to select their controls, but without a lot of detailed instruction and great care, it might be difficult to pick a representative sample. You should ensure that great care is taken over this aspect of the sampling to ensure the experimental results have meaning and generalisability to some larger well defined population.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Investigator CV		21 December 2012
Other: CV for Student		10 January 2012
Other: CV for Supervisor (Reilly)		
Other: Letter to Nursery Head/School Head Teacher	2	23 February 2012
Other: Invitation letter to Parent/Guardian	2	23 February 2012
Other: Study flowchart for school/nursery recruitment	1	23 February 2012
Participant Consent Form	1	10 January 2012
Participant Information Sheet: Parent	1	10 January 2012
Participant Information Sheet: Child	3	23 February 2012
Protocol	1	10 January 2012
REC application		06 January 2012
Response to Request for Further Information		23 February 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

<u>Reporting requirements</u>

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

<u>Feedback</u>

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.
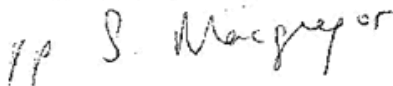
Further information is available at National Research Ethics Service website > After Review

12/WS/0015	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Gregory Ofili
Chair

Enclosures: "After ethical review – guidance for researchers"

Copy to: Dr Zubaida Alghaeed, Yorkhill Hospital
                Ms Joanne McGarry, R&D, NHS GGC

Appendix H

Paper published from this thesis

The Influence of Minimum Sitting Period of the ActivPAL^{TM} on the Measurement of Breaks in Sitting in Young Children

Zubaida Alghaeed[1], John J. Reilly[2], Sebastien F. M. Chastin[3], Anne Martin[4], Gwyneth Davies[1,5], James Y. Paton[1]*

1 School of Medicine, University of Glasgow, Glasgow, United Kingdom, 2 Physical Activity for Health Group, University of Strathclyde, Glasgow, United Kingdom, 3 School

of Health and Social Care, Glasgow Caledonian University, Glasgow, United Kingdom, 4 Institute of Sport, PE and Health Science, University of Edinburgh, Edinburgh,

United Kingdom, 5 National Heart and Lung Institute, Imperial College London, United Kingdom

Abstract

Background: Sitting time and breaks in sitting influence cardio-metabolic health. New monitors (e.g. activPAL™) may be more accurate for measurement of sitting time and breaks in sitting although how to optimize measurement accuracy is not yet clear. One important issue is the minimum sitting/upright period (MSUP) to define a new posture. Using the activPAL™, we investigated the effect of variations in MSUP on total sitting time and breaks in sitting, and also determined the criterion validity of different activPAL™ settings for both constructs.

Methods: We varied setting of MSUP in 23 children (mean (SD) age 4.5 y (0.7)) who wore activPAL™ (24 hr/d) for 5–7 d. We first studied activPAL™ using the default setting of 10 s MSUP and then reduced this to 5 s, 2 s and 1 s. In a second study, in a convenience sample of 30 pre-school children (mean age 4.1 y (SD 0.5)) we validated the activPAL™ measures of sitting time and breaks in sitting at different MSUP settings against direct observation.

Results: Comparing settings of 10, 5, 2 and 1 s, there were no significant differences in sitting time (6.2 hr (1.0), 6.3 hr (1.0), 6.4 hr (1.0) and 6.3 hr (1.6), respectively) between settings but there were significant increases in the apparent number of breaks - (8(3), 14(2), 21(4) and 28 (6)/h) at 10, 5, 2 and 1 s settings, respectively. In comparison with direct observation, a 2 s setting had the smallest error relative to direct observation (95% limits of agreement: -14 to +17 sitting bouts/hr, mean difference 1.83, p = 0.2).

Conclusion: With activPAL™, breaks in sitting, but not total sitting time, are highly sensitive to the setting of MSUP, with 2 s optimal for young children. The MSUP to define a new posture will need to be empirically determined if accurate measurements of number of breaks in sitting are to be obtained.

Introduction

With increasing recognition that adults and children spend most of their waking hours sitting, research on time spent sitting, and its impact on health has proliferated in recent years [1,2]. Evidence is also emerging that sitting periods interrupted frequently by periods of standing or activity may have different relationships with health outcomes than longer periods of uninterrupted sitting [3,4]. Animal studies provide supportive evidence that prolonged periods of uninterrupted sitting are related to increased risk of cardio-metabolic disease [1,3]. A recent review [5] concluded that the development of accurate methods for measurement of sitting time and breaks in sitting time was a high priority in sedentary behavior research.

Evidence from adults suggests that measurement of breaks in sitting may be less accurate with traditional accelerometers, such

as the Actigraph, than with accelerometers designed specifically to measure posture and posture transitions such as the activPAL™ [6]. The activPAL™ has been validated for measurement of both sitting and breaks in sitting in adults [7].

As yet there has been little research on the possible health consequences of variations between individuals in breaks in sitting time in children. This is largely because there has been a lack of practical, objective and validated methods suitable for measuring sitting time and breaks in sitting in free-living children [8]. Kwon et al [9] and Mitchell et al [10] both reported, in longitudinal studies of older children and adolescents, that Actigraph deter-mined breaks in sitting decline with age, but there is little evidence on the accuracy of the Actigraph for measurement of breaks in sitting [10]. Concurrent validity of the activPAL™ (against the Actigraph) for group-level estimates of total time sitting has been

established for pre-school [11] and older children [12]. Criterion validity (against direct observation) of activPAL[TM] measurements of time spent sitting was also shown to be high in our previous study of pre-school children [13]. However, evidence on criterion validity of the number of breaks in sitting is less clear and may depend on activPAL[TM] settings.

In adults, the available evidence points to more frequent changes in posture being generally beneficial for health [3,4]. Similar evidence is not yet available for children. However, the activPAL[TM] is an event-based monitor that samples at 10 Hz and could therefore capture very frequent changes in posture. It is not clear that events (postural changes) occurring at a frequency of

#1 HZ (i.e. #1 change per sec) are likely to have any physiological meaning. In order to screen out very short events, the activPAL[TM] software includes an algorithm that only counts events longer than a specified duration, set by default at 10 s minimum sitting/upright period (MSUP) (i.e. $10 s of sitting/ lying or upright data is needed to register as a new sitting/lying, or upright, event). In effect, this software setting determines the minimum period to define a new posture such as sitting [13,14]. In many published studies, this setting has been left at a default value of 10 s as per the manufacturer's specifications [15].

In a previous study in pre-school children, we changed the minimum sitting/upright period (MSUP) (which can be varied within the activPAL software from 1 s to 100 s) to 1 s because posture transitions appeared to be much more rapid in young children than in adults [13,14]. Using this 1 s setting, we found that the activPAL[TM] provided accurate relative rank-ordered assessments of breaks in sitting, but significantly overestimated the number of breaks in sitting when compared to direct observation [14].

It is easy to imagine that the time required to transition from one posture to another e.g. sitting to standing might be different at different ages. A young child would be expected to change posture very quickly but an elderly person might take much longer. However, at present, the optimum activPAL[TM] setting of a MSUP to define a change in posture for measurement of sitting time and breaks in sitting is not known, either for early childhood or later in childhood or adult life. Furthermore, the effect of changes in the minimum period of sitting/upright on measurement accuracy of both sitting time and breaks in sitting time is unknown and has not been explicitly investigated. The present research, therefore, aimed to examine the effect of variations in the activPAL[TM] minimum time setting on both the total time spent sitting, and breaks in sitting (study 1), and to determine the criterion validity of different minimum event duration settings (study 2) using direct observation as the criterion method.

Methods

Ethics Statement

The University of Glasgow Medical Faculty Ethics Committee approved the study. Parents gave written informed consented to participation and children assented to the individual study procedures.

We used two groups of children to investigate the effects of changing minimum sitting/upright period. For both studies age and sex were recorded, height and weight were measured and body mass index (BMI) calculated. They were converted to z scores using the appropriate 1990 British growth reference [16,17].

Study 1

For the first study, the data were collected from a convenience sample of 23 healthy, free-living preschool children in Glasgow, Scotland. Information letters were distributed to head teachers of nurseries (N = 4) and local contacts (mainly colleagues with pre-school aged children). Parents who agreed to take part made an appointment with the researcher where written consent was obtained and baseline data and measurements completed. Each child was asked to wear an activPAL[TM] monitor (PAL Technol-ogies, Glasgow, UK) continuously, 24 hrs a day, for between 5 to 7 days. The monitor was placed directly on to the skin of the child's mid-thigh area using a small hypo-allergenic adhesive gel patch (PALstickies[TM]), and was covered with a transparent sticky film (Tegaderm[TM]) to secure it. As the device used was not waterproof, parents were asked to remove the monitor for any showering, bathing or swimming during the monitoring period. It was not routinely removed during the night. Parents were asked to note in a daily diary any time the device was removed as well as the time the device was reattached. For each child, periods noted in the daily diaries when the child was not wearing the device e.g. because of swimming, bathing/showering or delayed reattachment because of forgetting were identified and excluded from the raw activPAL[TM] files before analysis. During the period of monitoring, the children were attending nursery during weekdays and were taking part in normal nursery activities – in the classroom, during physical activity in nursery school and during periods of free play. All parents received verbal and written information and instruc-tions about using the device before giving informed consent to the study.

For all children, the minimum duration of device wear time has been previously established as three weekdays with at least 6 hours of monitoring during waking hours per day [13]. In practice, in this study device wear time was much greater. In our final analysis, only weekdays were considered to avoid any effects arising out of different patterns of activity during weekend days.

Data Reduction, Operationalization of Sitting Variables

The activPAL[TM] output classifies an individual's activity into three categories: ''sitting/lying''; ''standing'' (standing with no movement); and ''walking'' (movement from one place to another). In addition, the activPAL[TM] identifies and counts posture transitions (sit-to-stand and stand-to-sit).

In this study, sitting (sit/lie) was characterized in the following ways:

1. Total time sitting. Waking time was defined from the first sit to stand transition in the morning, marking the fact that the child had woken. The researcher identified this transition by manual inspection of the event file produced using custom software (HSC PAL analysis software v 2.14) developed by by

Dall and Granat at Glasgow Caledonian University. This software allows detailed analysis of the activPAL[TM] output as classified by the original activPAL[TM] Professional Research Edition software. The software generates a file listing the time (in seconds) at which a change in output category (i.e. a transition) occurred [13]. Arbitrarily, the end of waking time was standardized at 9 pm for all participants on all days of measurement. Total time recorded as ''sit/lie'' during waking hours was calculated. We also calculated

the percentage of daily time spent in sit/lie and stand as recorded by activPAL[TM] during waking, as previous studies have included this as a measure of volume of sitting behavior [11].

2. Breaks in sitting. The number and frequency of interruptions (''breaks''), defined as the number of transitions recorded from ''sit/lie'' posture to ''stand'' [18] during waking time were counted using the activity profile (summarized by hour)

by activPAL[TM] Professional Research Edition software (Version 5.8.2.3). Only transitions from sit/lie to stand were counted and not stand to sit/lie transitions.

3. Sitting bouts. We calculated the number and duration of each individual sitting bout, defined as duration in seconds spent ''sit/lie'' ending in a postural transition [19]. The number and duration of sitting bouts (sit/lie) were quantified using HSCPAL analysis software (version 2.14). [13,14].

The length of individual sitting bouts and their distribution was represented by accumulation curves [19] and a fragmentation index [20]. Accumulation curves (Lorenz curves) characterize how an individual aggregates their sitting time [19,21] and relate the amount of time accumulated in bouts shorter or equal to a given length. These curves can be reduced to single metrics at any point along the curve but the 50% and 90% points have been suggested to be the most interesting [21]. The fragmentation index (calculated as the number of sitting bouts/total sitting time measured in hours) [20] is a metric that summarizes information about breaks and the accumulation curves in one single metric. The fragmentation index (with units of number of bouts/total sitting hr) normalizes the number of breaks in sitting by removing the influence of total sitting time and provides a simple single measure of whether an individual accumulates their sitting time in a many short bouts or in a smaller number of longer bouts [20]. A higher fragmentation index indicates that time spent sitting is more fragmented with shorter sitting bouts. Both these approaches have been used to characterize sitting behavior in adults [19,20].

As noted above, although the activPAL[TM] is an event based system, the analysis software only counts breaks in sitting lasting longer than a user defined MSUP in the new posture. This is intended to exclude very short postural ''events'' that are recorded by the monitor but are likely to have no physiological meaning, and is set by default at 10 s. In the present study, we systematically investigated the effect of reducing the MSUP from 10 s, through 5 s, 2 s, to 1 s within the activPAL[TM] software. Changing the MSUP in the activPAL[TM] software involves manually changing the setting in the range from 1 s to 100 s and only affects the time the monitor waits to decide whether a posture is seated or upright posture. Changing the MSUP has no effect on stepping time. Steps are detected directly using a different algorithm that does not take MSUP into account.

Study 2

The second study was an assessment of the criterion validity of measurement of breaks in sitting in a different group of free-living pre-school children. They were a convenience sample of 32 pre-school children (4.1 y (0.5)) recruited from nursery schools in Scotland who were videoed for an hour while playing freely at nursery while wearing an ActivPAL[TM] monitor. Data analysis was performed on children (n = 30) with a complete data set for activPAL[TM] and direct observation outcomes. The study is described in detail elsewhere [13,14] but in brief, each child wore an activPAL[TM] monitor and simultaneously was filmed for 1 hour during their usual activity in nursery. Second by second direct analysis of the video was then used to count number of breaks in sitting time.

In study 2, the raw activPAL[TM] files were reprocessed using MSUP of 2 s, 5 s and 10 s and for each child, the number and duration of sit/lie periods was calculated from direct observation files and was compared with the activPAL[TM] analyses using the varying settings.

Both studies used activPAL[TM] Professional Research Edition software (Version 5.8.2.3).

In both studies, the activPAL[TM] HSCPAL software files and the activPAL[TM] pal files (activity profile summarized by hour) were used in our data analysis. We made no use of the ''15 s epoch file'' in the available activPAL[TM] software.

Statistical Analysis and Study Power

Statistical analyses and calculations were conducted using the Minitab statistical software version 16.1 (State College, PA, USA) and MicrosoftH Office Excel 2007. For both studies, a convenience sample of around 20 children was deemed a priori, likely to be sufficient to characterize differences in the number of posture transitions, as measured between 1 s and 10 s minimum time spent sitting settings. Preliminary analysis of 20 sets of paired activPAL[TM] data (i.e. 10 s and 1 s data from the same child) in study 1 showed that the difference in number of posture transitions measured by the 10 s and 1 s setting was highly statistically significant and so only those children recruited to the study at that point were included and no further recruitment took place. Paired t tests were used to test the significance of differences in variables measured. Repeated measures Analysis of variance (ANOVA) using Tukey's correction for multiple comparison was applied to compare the mean values for each MSUP. A Bland-Altman analysis for assessing agreement between two measurements [22] was carried out. The limits of agreement between the number of sitting bouts during direct observation (criterion method) vs sitting bouts calculated by the activPAL[TM] using different MSUP activPAL[TM] settings (1 s, 2 s, 5 s and 10 s minimum sitting/ upright period) were set at mean difference $+/2 1.96$ x standard deviation (SD). (The graph for the 1 s comparison has previously been previously published [13]). The pattern of accumulation of sitting bouts by direct observation data and activPAL[TM] data with different settings (1 s, 2 s, 5 s &10 s) was represented by accumulation curves [19]. All variables were checked for normal distribution and means and SDs were used to summarize normally distributed values. For all tests, significance was taken at p = 0.05.

Results

Characteristics of Study Participants

Study 1. Of the 23 children recruited to study 1, 20 provided adequate data of at least 3 days (3 children wore the monitors less than 3days during the study) [11], 9 boys and 11 girls; mean age 4.5 (SD 0.7); mean height 107.7 cm (4.9), mean weight 19.6 kg (3.9) and mean body mass index 16.6 kg/m^2 (2.0). The mean z-scores were 0.24 for height, 0.60 for weight and the median z-score 0.16 for body mass index (BMI). Mean (SD) monitoring time was 3.8 d (0.7), 22.3 hr (1.5) per 24 hr period, of which a mean of 11.9 hr (1.0) was in waking hours. Missing data (where monitor was removed because of swimming, bathing/showering or monitor and not reattached according to parent's record) accounted for a mean of 5.1% (SD 3.4) of total monitoring hours.

Breaks in Sitting in Free-living Children

Study 1. A plot of number of breaks in sitting per hour against time during 24 hours is shown in figure 1 for both day and night hours using 10 s vs 1 s MSUP. There was a gradual increase in the number of breaks per hour from morning until afternoon with a dip after lunch-time and a peak at around 4 pm followed by a decrease in the evening until the child went to sleep (Figure 1). During the night, generally no breaks were recorded from midnight until early morning. However, occasionally a few breaks occurred between 9 pm and 12midnight (Figure 1).

In the light of the above, the rest of the analysis was restricted to breaks in sitting during waking hours. Using a minimum
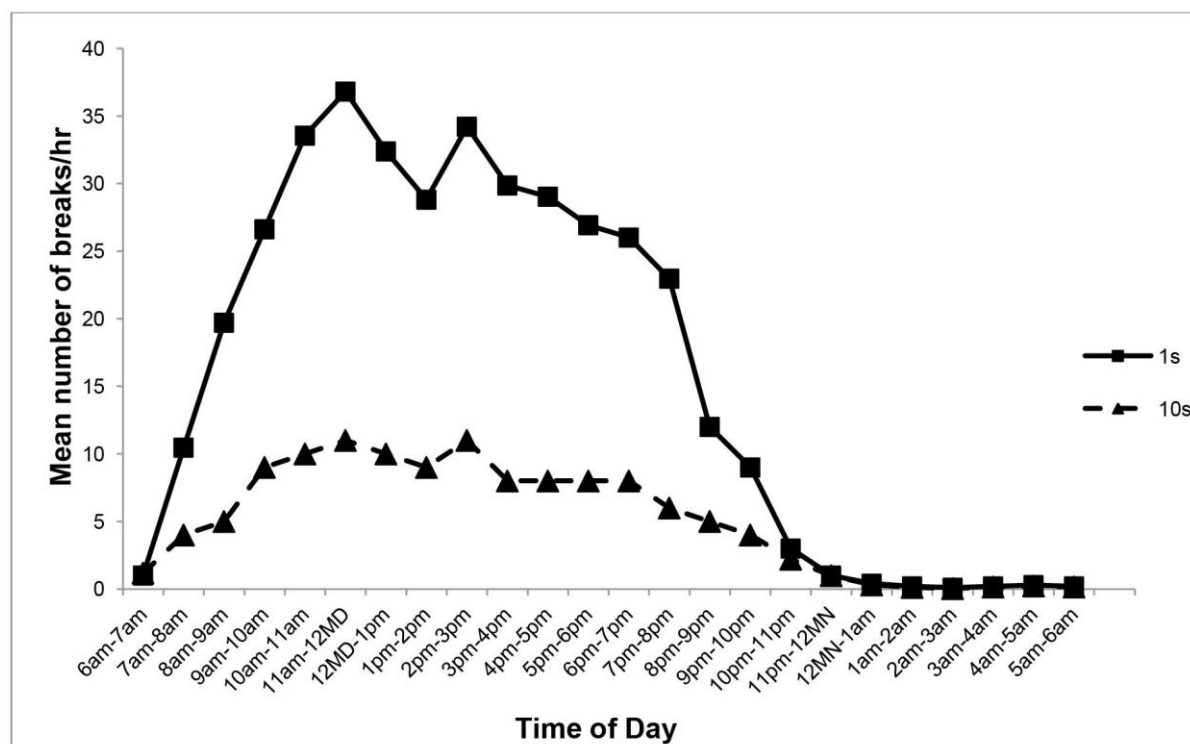
Figure 1. The mean number of breaks in sitting/hr during 24 hr using minimum time to define new position settings of 10 s and 1 s. Study 1(n = 20).

activPAL[TM] sitting/upright period of 10 s, the mean (SD) percentage of waking time spent sitting was 52.3 (6.2) %. The total sitting time was 6.2 hr, (1.0) during waking hours (11.9 hr (1.0)). The total number of breaks in sitting during waking hours was 109 (18) giving a mean number of breaks of 8 (3) per hour. Using a 10 s MSUP, around 90% of sitting bouts during waking hours were #8 min (1.5) and the mean (SD) fragmentation index

(number of bouts/total sitting time (hr)) [20] during waking hours was 19.3 (3.7) (Table (1).

The Difference in Estimated Sitting Time and Breaks in Sitting using 10 s vs 1 s MSUP

Study 1. The measures of sitting time during waking hours with the different MSUPs are shown in Table 1. There were no significant differences in the mean sitting time when expressed

Table 1. Description of sitting behaviors during waking hours[+], mean (SD) for study 1 (n = 20).

	10 s setting (Default) Mean (SD)	5 s setting Mean (SD)	2 s setting Mean (SD)	1 s setting Mean (SD)	p-value
Total sitting time (hr)	6.2(1.0)	6.3(1.0)	6.4(1.0)	6.3(1.6)	0.9
% Sitting time (defined as-sit/lie only)	52.3 (6.2)	52.5 (5.9)	53.5 (5.4)	52.9 (6.3)	0.7
% Sitting time (defined as sit/lie and quiet standing)	80.1(8.3)	80.3 (4.6)	82.1 (3.9)	81.5(8.9)	0.5
Number of breaks (sit to stand)/hr*	8 (3)	14 (2)	21(4)	28 (6)	0.00
Total number of breaks in sitting (transitions)*	109 (18)	173 (43)	278 (78)	376(90)	0.00
Number of sitting bouts[&]	118 (18)	182 (28)	289 (52)	382 (80)	0.00
50% sitting bout length	80 s (14.7)	55 s (4.2)	50 s (4.2)	42 s (7.7)	0.00
90% sitting bout length	8 min (1.5)	6 min (1.1)	3 min (1.0)	60 s (10.4)	0.00
Fragmentation index	19.3 (3.7)	29 (5.0)	46 (9.0)	61.6 (16.4)	0.00

[+]Waking hours were defined as ''From the first sit to stand transition in the morning to 9 pm''.
*Calculated from activity profile summarized by hour using activPAL[TM] Professional Research Edition (Version 5.8.2.3).
[&]Calculated using activPAL[TM] HSCPAL analysis software (version 2.14). The PAL files generated by the activPAL[TM] software were imported into HSC PAL analysis software (developed by Dall and Granat).

either as total time measured in hours or as a percentage (6.2 hrs (52.3%) vs 6.3 (52.9%), Paired t test p = 0.45) (Table 1).

However, for the number of breaks, number of bouts, bout periods and fragmentation index (number of bouts/sitting hour) there were significant differences as the MSUP was varied. Changing from a 10 s setting to a 1 s setting for MSUP led to significant increases in: the total number of breaks in sitting (109 (18) vs. 376 (90), p = 0.001); the number of breaks per hour (8 (3) vs. 28 (6), p = 0.0001); and the total number of sitting bouts (118 (18) vs. 382 (80), p = 0.0001). Around 90% of sitting bouts were #8 min using a 10 s setting but were #1 min using a 1 s setting. The fragmentation index using a 1 s setting was nearly 3 times greater than when using 10 s setting: (61.6 (16.4) vs. 19.3 (3.7), p = 0.001) consistent with more fragmented, and shorter sitting bouts.

In addition, marked inter-individual differences in the pattern of accumulation of sitting bouts were observed. As an example, in 2 children, where the total time sitting of both children was similar at 53% and 55% of 10.2 h (0.9) and 10.7 h (1) waking hours respectively, the accumulation of their bouts was different: using a 10 s minimum sitting/upright period, 90% of sitting bouts were
#6 min in child 1 and #15 min in child 2 and about 50% sitting bouts were #55 s in child 1 and #75 s in child 2. Moreover, child 1 had a fragmentation index of 17.9 (5.2) and 82.5 (20) with 10 s and 1 s settings respectively, and child 2 had a fragmentation index of 15.8 (4.2) and 37.3 (10.2) with 10 s and 1 s respectively.

Study 2. Thirty preschool children completed simultaneous activPAL[TM] and direct observation monitoring (10 boys and 20 girls; mean age 4.1 y (0.5), mean height 105.1 cm (5.1), mean weight 18.7 kg (3.8) with a mean body mass index 16.8 kg/m2 (2.1). The mean z-scores were 0.64 for height, 0.79 for weight and 0.60 for BMI. A total of 16167 s (14.2%) was 'off screen' time from 113,917 total measured seconds for the 30 children [13,14].

In study 2, combining data from all participants (n = 30), the total time spent sitting during direct observation were compared with the total time spent sitting using the activPAL[TM] setting 2 s, 5 s and 10 s MSUP. The total time spent sitting was 12.5 hr during direct observation and 11.3 hr with 1 s MSUP [14]. With 2 s, 5 s and 10 s the total sitting time was 11.4 hr, 11.2 hr and 11.3 hr, respectively.

For bouts of sitting, the average number of bouts per hr using direct observation was compared with bouts measured simulta-neously using the activPAL[TM] using 2 s, 5 s and 10 s MSUP respectively. Figure 2 shows Bland-Altman plots comparing the different numbers of sitting bouts during direct observation vs different MSUP of 1 s, 2 s, 5 s and 10 s on the activPAL[TM] for each child (n = 30) are shown. It can be seen that the use of a 2 s setting for activPAL[TM] MSUP minimized bias and showed no significant difference relative to direct observation (limits of agreement - 14 to +17 bouts per hr, mean difference 1.83, paired t-test p = 0.2). However, the 5 s and 10 s settings underestimated the number of sitting bouts as measured by direct observation (for 5 s limits of agreement -23 to 8, mean difference -7.27 and for 10 s limits of agreement -29 to 4, mean difference -12.57, paired t-test p = 0.001, respectively). While the bias is much smaller with a 2 s setting the limits of agreement are quite wide, and of similar magnitude to the other settings. This means that the average with a 2 s setting will be more accurate, but for any individual the errors with 2 s will be nearly as large as for the other settings.

Figure 3 shows the pattern of accumulation of sitting bouts during direct observation with 1 s, 2 s, 5 s and 10 s MSUP. 90% of sitting bouts were identical (at #2 min) for both direct observation and from the activPAL[TM] using the 2 s MSUP.

Discussion

Main Findings and Study Implications

This is the first study to examine the effect of varying the activPAL[TM] MSUP to define a new posture setting on measure-ments of total time spent sitting and breaks in sitting. In study 1 we showed that varying the activPAL[TM] setting had only a negligible impact on measurement of total time spent sitting. However and in contrast, for breaks in sitting there is a marked difference varying systematically with the setting used.

In study 2, we showed that the systematic differences in measures of breaks in sitting described in study 1 have an impact on the accuracy of the measurement of breaks in sitting. The result is that a default setting of 10 s for the activPAL[TM] appears unsuitable for quantification of breaks in sitting in young children, in whom a minimum sitting/upright period of 2 s will provide much higher accuracy with minimal bias.

The present study also shows that important features of sitting behavior in young children can be characterized objectively by a few fundamental metrics: volume of sitting; frequency of breaks in sitting; and pattern of accumulation of sitting bouts as represented by accumulation curves and a fragmentation index [21]. Using a 2 s MSUP, the mean volume of sitting in study 1 was 6.4 hr (1.0) during waking hours, the number of breaks in sitting around 21/hr (4), the fragmentation index 46(9.0), and 50% of sitting bouts were less than 50 s (4.2) (Table1).

Healy et al [4] have previously reported in adults that increased breaks in sitting time (resulting in short sitting bouts) are associated with better metabolic health, a relationship that was independent of total sitting time. The fact that sitting behaviors can be characterized objectively by a few simple measures means that comparative studies investigating the longer-term health effects can now be undertaken in children.

Comparisons with Other Studies

To our knowledge, no previous studies have examined the effect of the activPAL[TM] MSUP to define a new posture on measurements of sitting time and breaks in sitting.

In general, other studies have not commented on what setting was used to define minimum sitting/upright period. As an example, Lyden et al in a recent activPAL[TM] validation study (against direct observation) of 13 free-living adults monitored for about 10 consecutive hours on 2 separate days [4 M, 9 F; mean age 24.8 (5.2)] reported that the activPAL[TM] was a suitable tool to measure breaks in sitting in this older age group with 5.1 (range 2.8–7.1) breaks in sitting per sitting hour [6]. This study did not specify whether the default 10 s MSUP was used.

Harrington et al noted the mean length of sitting bouts in adolescent females using activPAL[TM] was 9.8 (0.2) minutes [18]. Harrington used a customized MATLAB programme to process the activPAL[TM] data output files. This examined each epoch which contained a full 15 s of sitting/lying and classified this as the beginning of a sitting bout which continued until the next 15 s bout of standing or stepping was identified. Chastin and Granat using the activPAL[TM] with a 10 s MSUP found that the mean sitting bout length in free-living adults was 45 minutes [19]. In contrast, and using a 10 s minimum sitting time for purposes of comparison, the majority of sitting bouts for the young children in the present study (study 1) lasted #8 minutes suggesting that the children studied predominantly accumulated their sitting time in short bouts.

Studies using objective measures of fragmentation index are non-existent in children and scarce in adults. A recent study in 30 healthy adults (using activPAL[TM] continuously over 7days) found
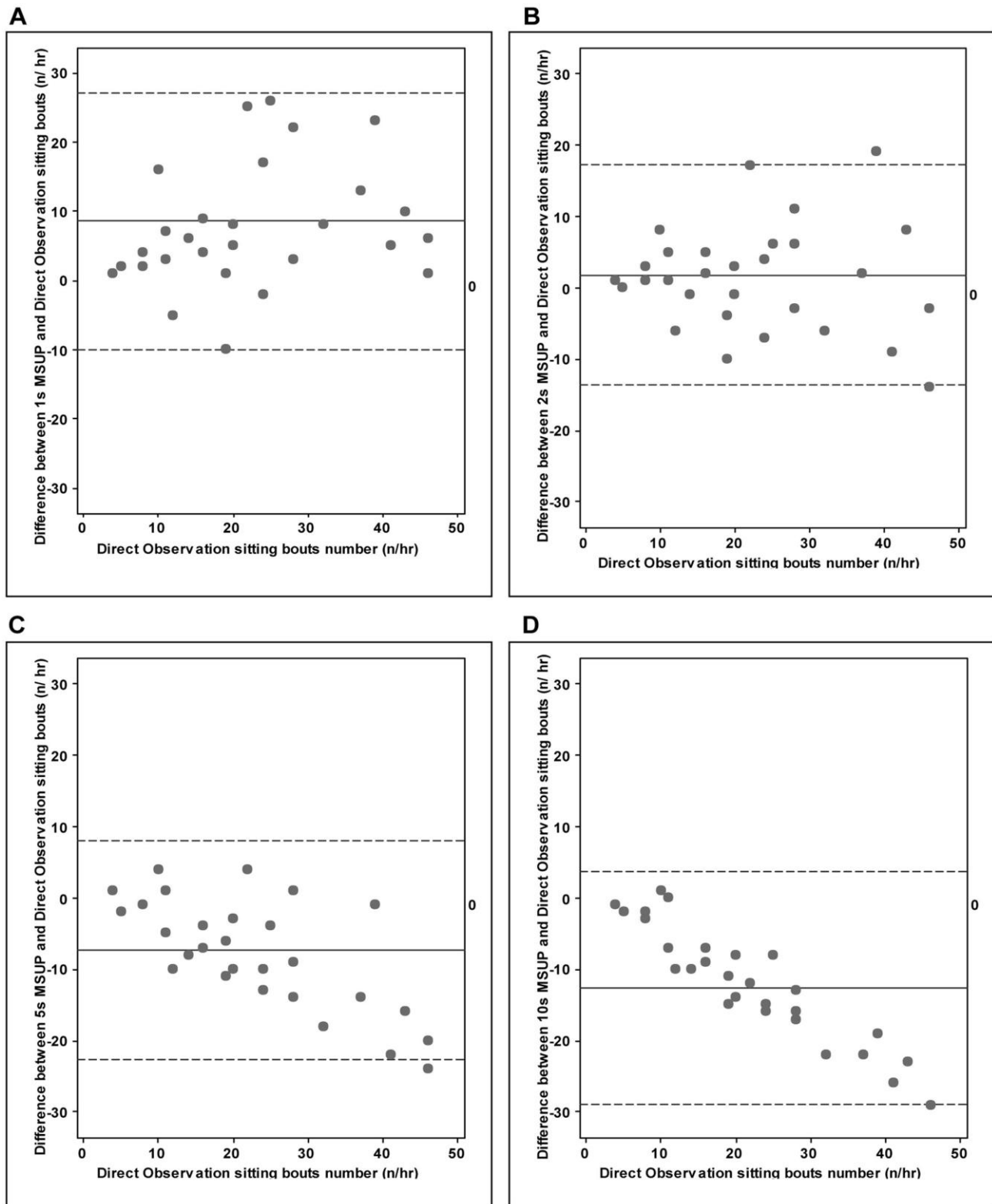
Figure 2. Individual Bland-Altman plots comparing the difference in number of sitting bouts during direct video observation (direct observation) with the number of sittings bouts measured by the activPAL^{TM} with different activPAL^{TM} settings for minimum sitting/upright period (1s – diff1 (A), 2s – diff2 (B), 5s - diff5 (C) and 10s - diff10s (D)). Study 2 (n = 30). Data for 1 s taken from Davies et al [15]. Direct Observation is considered the criterion or gold-standard and it is used on the x-axis. Mean bias is represented by a solid line, 95% limits of agreement by dashed lines.
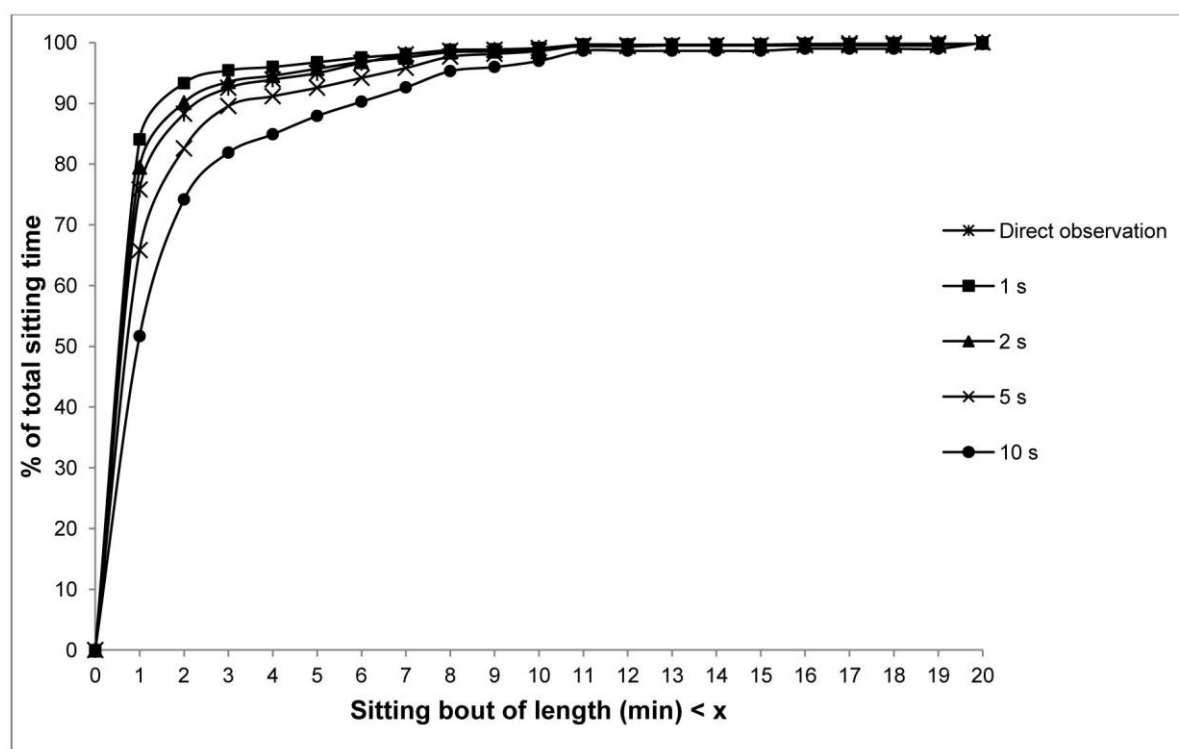
Figure 3. The pattern of accumulation of sitting bouts during direct observation, using minimum sitting/upright periods of 1s, 2s, 5s and 10s. Study 2 (n = 30). The numbers on the x-axis are cumulative – hence the y intercept at a particular x value represents the number of sitting bouts occurring shorter than or equal to a given x axis value.
doi:10.1371/journal.pone.0071854.g003

that the mean fragmentation index [bouts/sitting time (including sleeping time) (hr)] in men was 2.6(0.8) and 3.3(0.4) in women [20]. In the present study, the mean (SD) fragmentation index (again using the default 10 s MSUP for comparison) was much higher, 19.3(3.7). Our present study is not directly comparable because we excluded sleeping time, where subjects would be expected to have no postural transitions. A preliminary reanalysis of 3 subjects in the present study, chosen at random, showed that even including sleeping time the fragmentation index is about 3 times greater than that reported by Chastin et al. Our evidence, therefore, suggests that young children have much more fragmented sitting time with a pattern of shorter sitting bouts interrupted by more frequently by breaks.

Because of its impact on the measurement of breaks in sitting and other measures such as fragmentation index, the present study suggests that more attention must be paid to this instrument setting. It seems intuitively likely that the most suitable setting for measurement of breaks in sitting time may vary with age. We would hypothesize that children can transition to a new posture more frequently than adults, and the optimum setting for measurement in breaks in sitting may lengthen as subjects get older. We suspect that it is likely that empirical studies using the activPAL™, or other similar event based monitoring systems, will in future be required to define the best setting for minimum duration of sitting for each age.

Study Strengths and Limitations

The present study does not assess the biological importance of sitting time or fragmentation, but that was not the aim of the present study. Methodological evidence aimed at the establish-ment of accurate yet simple and objective measures for charac-

terizing sitting time and fragmentation will be fundamental to future studies which try to relate these constructs to health outcomes, and essential for evaluation of future intervention studies.

Previous studies of movement in young children and adults, particularly those using the Actigraph monitor, have used an analytical approach based on the analysis of sitting in 15 s epochs [12,18]. A detailed comparison of the impact of different MSUPs in an event based analysis, as in our study, vs a 15 s epoch approach is beyond the scope of this paper. However, a preliminary analysis of 10 files from study 2 using the 15 s epoch file analysis present in the activPAL™ software showed that there were few changes when we used different MSUP settings and these were statistically not significant for either total sitting time or number of sedentary bouts.

Conclusion

This study has established that the setting of MSUP to define a new posture has a significant impact on measurement of breaks in sitting in young children but not the measurement of total sitting time. In the age group we studied, 2 s appears to be an appropriate minimum sitting/upright period to define breaks in sitting using the activPAL™. It is probable that the optimum instrument setting for minimum sitting/upright period will be different at different ages. Standardization of the technical aspects of measurement and of measures to describe sitting time will allow longer term studies of the health effects of sitting behaviors as well as providing comparable baseline data for intervention studies

Author Contributions

Conceived and designed the experiments: ZA JJR SFMC JYP. Performed the experiments: ZA AM GD. Analyzed the data: ZA JJR SFMC GD JYP. Wrote the paper: ZA JJR SFMC GD JYP.

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