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Habitual Physical Activity Assessment Using Objective Measuring Devices: Observations in Lean and Obese Adults and Children

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DECLARATION

I declare that this thesis was composed by myself and that all the data were collected and analysed by myself. Neither the thesis nor the original work therein has been submitted to this or any other institution for a higher degree.

Signature …….

Date ……

ABSTRACT

Physical inactivity is one of the major public health problems in many parts of the World. In Scotland it is reported that two thirds of Scottish adults (>16yrs) and one third of Scottish children (≤16yrs) do not do sufficient physical activity to gain the health benefits of physical activity. Furthermore, there is still much debate about the nature and volume of physical activity required to provide health benefits. Therefore, more investigations are required to help improve our understanding of the links between physical activity, obesity and health. In addition, the assessment of habitual physical activity needs to be accurately quantified using appropriate methods that are valid and reliable.

The main aims of this thesis were thus to assess the validity and reliability of three new generations of movement sensing devices (Actigraph, ActivPAL and SenseWear PRO_2) in adults and adolescents in a controlled laboratory environment and to then use the most valid and reliable device in assessing the habitual physical activity of adults (lean and overweigh/obese) and adolescents in a free-living situation. Following objectively assessing the habitual physical activity, investigation of the associations between physical activity status and cardiovascular and metabolic disease risk markers in adults and adolescents were the last main aims of this thesis.

In the first study, the results indicated that the new generations of the three devices were reliable in assessing EE during walking on the flat and on a 5% incline in lean and overweight/obese adults and lean adolescents. However, none

of these devices and the methods or programme versions that were selected and applied was able to accurately estimate EE during walking on a treadmill. However, based on the sensitivity data obtained and previous evidence, the Actigraph was considered the most appropriate device for assessing the habitual physical activity due to its ability to discriminate between physical activity intensities.

The second and third studies concluded that adults (including lean and overweight/obese) met the recommended physical activity guidelines for health and wellbeing purposes. However, the data suggest that overweight/obese participants may need to be advised to spend more time in MVPA and probably more vigorous activity to not only reduce body fat but also to increase cardiorespiratory fitness and reduce their chances of future cardiovascular and metabolic disease.

The fourth and fifth studies, demonstrated that the Scottish adolescents –in the cross sectional study- were below the recommended physical activity guidelines. When the method of physical activity assessment was adjusted the Scottish adolescents were similar to the adolescents in other European countries and were observed to be more active than adolescents in some of the developed countries such as American adolescents (Texas State). In the case of lean adolescents who have a low physical activity- but who are not sedentary- the cardiovascular and metabolic disease risk markers may not be obvious at this stage, but the differences in glucose and HOMA-IR suggest that there may be early signs of progression towards metabolic disease in this group.

The importance of the associations between vigorous physical activity and body fat, as well as between body fat and the risk markers of metabolic disease, suggests that future intervention studies should focus on monitoring the outcome from vigorous physical activity interventions vs. moderate activity within current guidelines.

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GLOSSARY OF TERMS

The following abbreviations have been used frequently throughout the text of this document.

ACSM: American College of Sports Medicine

AG: ActiGraph

AHA: American Heart Association

ANOVA: Analysis of Variance

AP: ActivPAL

ARA/EPA: Aarachidonic Acid / Eicosapentaenoic Acid

BF%: Body Fat percentile

BMI: Body Mass Index

BP: Blood Pressure

CDC: Centers for Disease Control

CHD: Coronary Heart Disease

CPM.b^{-1}: Count Per Minute per Beat

CPM: Count Per Minute

CRP: C-Reactive Protein

CV: Coefficient Variation

CVD: Cardiovascular Disease

DHHS: Department of Health and Human Services

DLW: Double-Labeled Water

ELISA: Enzyme-Linked Immunosorbant Assay

FFA: Fat Free Acids

FFM: Fat Free Mass

HDL: High Density Lipoprotein

HEPS: Health Education Population Survey

HOMA-IR: Homeostasis Model Assessment- Insulin Resistance

HR: Heart Rate

hsCRP: high selectivity C- Reactive Protein

HUFA: Highly Unsaturated Fatty Acid

IC: Indirect Calorimetry

ICC: Interclass Coefficient Correlation

IL-6: Interleukin-6

IOM: Institution Of Medicine

Kcals.min^{-1}: Kilocalories per Minute

LDL: Low Density Lipoprotein

MAP: Mean Arterial Pressure

MET or METs: Metabolic Equivalent

MVAP: Moderate to Vigorous Physical Activity

n-3: Omega 3

n-6: Omega 6

NCEP-ATPIII: National Cholesterol Education Programme- Adults Treatment Panel III

NHANES: National Health and Nutrition Examination Survey

PAEE: Physical Activity Energy Expenditure

PAR-Q: Physical Activity Readiness- Questionnaire

SD: Standard Deviation

SHeS: Scotland Health Survey

SW: SenseWear PRO$_2$

TNF-α: Tumor Necrosis Factor- alpha

Vigo.: Vigorous

VLDL: Very Low Density Lipoprotein

VO_2: Volume of Oxygen in expired air

VO_{2MAX}: Maximum Oxygen uptake

Vvigo.: Very vigorous

Chapter 1. Introduction and literature review

Sedentary leisure time activity such as watching TV, using of computer and using some communication technology (e. g. chatting using mobile phones) may displace sufficient physical activity (Five-year review of 'Let's Make Scotland More Active', 2009). As a result, health status and wellbeing may be affected negatively. Therefore, some countries such as Scotland set up a long term strategy to face this problem. Assessment of physical activity using objective measures is one of the information sources that may provide better estimation about population health status and, in turn, provide strong evidence to the policy and decision maker to improve the need of changes in both policy and culture. On the other hand, some of physical activity recommendations and guidelines are updated during the last decade for adults and children by different health and medical organizations (Pate *et al.*, 1995; Haskell *et al.*, 2007). More investigation may be needed to provide information about physical activity status and the relationship between physical activity status and some chronic diseases such as cardiovascular and metabolic diseases.

1.1 Physical activity

Physical activity has been defined as body movement, produced by skeletal muscles, resulting in energy expenditure (Caspersen *et al.*, 1985). The measurement of physical activity is based on duration, intensity and frequency. Duration means the time spent in certain types of activity. Intensity means the level of the physical effort required and has been used to classify physical

activity into light, moderate, vigorous and very vigorous intensity bands. Frequency is the number of times physical activity is repeated and is usually measured per week. The type of physical activity can be classified as weight bearing physical activity such as carrying your own body weight (e. g walking) or free-weight training (e. g lifting weight), or non-weight bearing such as swimming, cycling and riding a horse.

The health benefits of physical activity include physical, psychological, sociological and mental benefits (Blair and Brodney, 1999; Wankel and Berger 1990; Fox, 1999). For health purposes, the recommended guidelines of physical activity for adults, children and adolescents are regularly updated by government and non-government heath organizations (Pate *et al.*, 1995; Catto *et al.*, 2009; Strong *et al.* 2005; Haskell *et al.*, 2007; U.S. Department of Health and Human Services, 2008; Currie *et al.*, 2004). For example, the recommended physical activity guidelines for adults (18-65) are: $\geq$30minute (duration), $\geq$moderate (intensity), $\geq$5d.wk^{-1} (frequency) (Catto *et al*., 2009). In children and adolescents, the recommended physical activity is higher as they are advised to engage in: $\geq$60minute (duration), $\geq$moderate (intensity), $\geq$5d.wk^{-1} (frequency) (Strong *et al.,* 2005; Currie *et al*., 2004). There are more than 30 different methods of assessing physical activity including objective and subjective methods (McArdle *et al.*, 2010). One of the most common objective physical activity measures is to use an accelerometer device (more details are available in section 1.2). The advantages of using accelerometer device are summarised in Table 1.1.

Table 1.1 Some of the advantages of using accelerometer device in assessing habitual physical activity.

Characters	**Advantages**
Data collection	Collect data during a week or more (e. g. 14 days)
Storage data	≥1 MB of non-volatile flash memory
Reliability and accuracy	Reliable and accurate in assessing individual activity levels
Size and weight	Small and light (about 45gm)
More features	Most of them are provided with easy to use software, direct USB connection, and a rechargeable battery.

In Scotland as a nation, physical inactivity is one of the major public health concerns as two thirds of Scottish adults and one third of Scottish children do not do sufficient physical activity to gain the health benefits of physical activity (Five-year review of 'Let's Make Scotland More Active', 2009). As a result, the Scottish Government established the "Let's Make Scotland More Active" initiative (LMSMA) in 2003. LMSMA is a long-term strategy and aims to achieve at least 50% of all adults aged over 16, and 80% of all children aged 16 and under meeting at least the minimum recommended guidelines of physical activity by 2022. Therefore, more investigations are required in order to understand and improve upon the habitual physical activity habits of the population and, in turn, to explore the implications for the public health of the Scottish population.

1.2 Physical activity and health benefits

The benefits of physical activity have been explored in terms of health promotion and in terms of chronic disease prevention for healthy people, people at risk of some chronic diseases, and people with chronic diseases (Lee *et al.*, 1999; Farrel *et al.*, 2007; Blair *et al.*, 1999; Ekelund *et al.*, 2009). In a review of literature, Blair and colleagues (1999) concluded that physical inactivity and low cardiorespiratory fitness play roles as mortality predictors as well as overweight and obesity.

Over the last decades the prevalence of overweight and obesity has increased dramatically (Visscher *et al.*, 2002), and this is thought to be partly related to lower levels of physical activity (Jebb and Moore, 1999). Recent evidence suggests the importance of being physically active from an early age such as in childhood. Ekelund and colleagues (2009) concluded that even small increases in physical activity may significantly reduce the risk of metabolic syndromes in healthy children. It has been suggested that physical activity in early age such as in childhood and adolescence may help not only to improve choice of a healthy lifestyle in adulthood but will also act to reduce the risk of many cardiovascular and metabolic diseases (Mealey, 2008). Furthermore, the benefit of physical activity in childhood can carry over into adulthood as active children are more likely to be active as adults (Currie *et al.*, 2004). A recent study by Martins and colleagues (2010) concluded that even among overweight adolescents, participants with higher cardiorespiratory fitness were at lower prevalence of cardiovascular disease risk factors. This highlights the complex interaction between physical activity, adiposity and risk reduction that is not yet

fully understood. Given all these findings, the question is: do the public people in Central Scotland engage in a beneficial amount of physical activity for health promotion and is this enough for cardiovascular and metabolic disease risk prevention?

To answer this question, habitual physical activity needs to be assessed using an appropriate method and with valid and reliable objective measures such as accelerometry and heart rate (HR) monitoring.

1.3 Validity and reliability of accelerometry

There are a number of accelerometer devices that have been manufactured and developed to assess physical activity (Schmidt *et al.*, 2003). However, few of these instruments are reliable and valid devices for assessing physical activity, and some of them have not been examined for use in children and adolescents nor comparing obese/overweight versus lean apparently healthy adults (Strath *et al.*, 2003; Vogels *et al.*, 2007). When accelerometry devices are used, there are number of challenges that may contribute to inappropriate assessment of physical activity or energy expenditure (EE). Therefore, the numbers of studies that have examined the validity, reliability, and development aspects of different types of accelerometer devices have been increased during the last decade (Strath *et al.*, 2003). Troiano (2005) concluded that some accelerometer devices provide information of overall estimation of body movement but a fundamental research challenge has been to determine how counts per minute (CPM) provided by accelerometers link to more meaningful

indicators such as activity EE or time spent in different physical activity intensities such as light, moderate and vigorous activity. Moreover, accelerometers have been found in some studies to overestimate light intensity of physical activity and underestimate vigorous intensity of physical activity (King *et al.*, 2004; Schmidt *et al.*, 2003).

One of the reasons behind these challenges is that accelerometer devices are based on biomechanical principles while physical activity effort and energy expenditure are physiological and biomechanical based. For example, Cole and colleagues (2000) demonstrated that growth and maturation of children and adolescents plays a role in perturbation of the results of some studies. Thus, valid and reliable assessment of physical activity has become a big challenge when accelerometry devices are used by exercise scientists, physiologists, epidemiologists, and clinicians. Recent studies have suggested that continued investigations on validity and reliability of different types of accelerometers are needed especially the new and developed generations of devices (Brage *et al.*, 2003; Chen, and Bassett, 2005; Freedson *et al.*, 2005). Thus, validity and reliability evaluation of some of the new generation of the available accelerometer devices that are used to assess activity EE and physical activity intensity may need to be investigated especially in populations with different ages and body composition.

The number of studies that have assessed physical activity using objective measures such as pedometer, accelerometry or heart rate monitoring increased about tenfold during 2004 to 2005 compared to the previous decade between 1994 and 1995 (Janz, 2006). As a result, more types and generations

of accelerometry started to be evaluated for validity and reliability. Since 1997 the numbers of studies that have examined the validity and reliability of different types of movement sensing devices including Actigraph (AG), ActivPAL (AP) and SenseWear (SW) have increased (Strath *et al.*, 2003) and the next sections of this introduction will provide a brief overview of the work completed using these devices.

1.3.1 ActiGraph (AG)

ActiGraph (AG) is one of the most widely used accelerometry devices for assessing physical activity (PA) and is currently used in the US National Health and Nutrition Examination Survey (NHANES IV) (Crouter *et al.*, 2006b). AG is a uniaxial accelerometer measuring vertical accelerations and formally known as Computer Science and Applications (CSA) from Manufacturing Technology Inc. (ActiGraph, LLC, Fort Walton Beach, FL, USA).

The new generation of AG (GT1M) is a small device (51x50x1.6cm) and lightweight (~45 g) device. More technical specifications of the device are described elsewhere (Crouter *et al.*, 2006b). It is designed to be worn on the right side of the hip. The data provided by AG for physical activity assessment includes counts per minute (CPM), energy expenditure estimation ($Kcals.min^{-1}$) and step counts. CPM as raw data has been used to develop the counts cut-off points of each physical activity intensity and to estimate physical activity energy expenditure PAEE in adults. The validity and reliability of AG

has been examined in terms of physical activity energy expenditure (PAEE) (Crouter *et al.*, 2006a; Freedson *et al.*, 1998; Puyau *et al.*, 2002; Freedson *et al.*, 1997; Matthews, 2005; Trost *et al.*, 2002). In turn, a number of different equations have been developed to estimate PAEE from the raw counts that are obtained from AG.

One of the common equations used to estimate PAEE (Kcals.min^{-1}) from AG is Freedson's equation for lean adults (Freedson *et al.*, 1998). Freedson and colleagues (1998) developed the regression equation on a sample of 35 lean young adults (23.8±4yrs) including males and females. Participants performed 2 walking speeds (4.8, 6.4Km.h^{-1}) and 1 running (9.7Km.h^{-1}) speed on a treadmill. The equation was subsequently cross validated on the remaining sample of 15 participants. Across all speeds, the result showed excellent correlation between actual and predicted EE from AG using the developed equation (r=0.93, SEE=±0.93kcal.min^{1}, P<0.05). The developed Freedson's equation is: Kcal.min^{-1} = (0.00094 x CPM) + (0.1346 x body mass (Kg)) – 7.37418 (Freedson *et al.*, 1998).

Freedson's equation was developed using a previous model of AG (Actigraph MTI Model 7164) (Freedson *et al.*, 1998). In a recent study, Slootmaker *et al.* (2009) examined the validity of the AG model 7164 to assess PAEE in lean adults using Freedson's equation during walking on the flat using a treadmill and walking up and down stairs (Slootmaker *et al.*, 2009). They found that AG with using Freedson's equation underestimated energy expenditure during walking at 3km.h^{-1} on the flat by 44% and by more than half of the measured

EE (using indirect calorimetry) (>50%) during walking up stairs at 80 & 100 steps/min.

On the other hand, Matthew *et al.* (2005) reviewed a number of studies that calibrated AG accelerometers during different physical activities including walking and running. They concluded that counts per minute (CPM) of AG increased with an increase in ambulatory speed which indicates that AG is a strong objective measure for intensity of dynamic physical activity (Matthew *et al.*, 2005). Although some PAEE ($Kcals.min^{-1}$) as a result of physical activities are unlikely to be accurately measured by AG such as static exercise or weight-lifting, AG appears to be an adequate instrument to assess physical activity intensity levels (e.g. light, moderate, vigorous) (Kai *et al.*, 2003). However, Freedson's equation has been developed for AG model 7164 to predict PAEE ($Kcal.min^{-1}$) in lean young adults. This equation may need to be examined in the new generation of AG (GT1M) in lean adults and to investigate whether it is applicable in overweight/obese adults and lean adolescents during different walking speeds. Therefore, one contribution of the current thesis will be to apply the Freedson's equation on the new generation of AG (GT1M) to estimate PAEE for lean and overweight/obese adults and lean adolescents during different walking speeds on the flat and on a 5% incline compared to assessment of energy expenditure using indirect calorimetry (IC).

1.3.2 ActivPAL (AP)

The second selected type of movement sensing device is ActivPAL^{TM} series 6.2 (AP). AP accelerometer is manufactured by PALtechnologies Ltd. Glasgow, Scotland. It is 5.4cm (L) x 3.5cm (W) x 0.6cm (D) and designed to be worn at one third the distance between the hip and the knee on the midline of the thigh as it responds to gravitational acceleration and posture can be classified as sitting/lying, standing and walking or running via the inclination of the thigh. This device attached to the skin using PALstickies. PALstickies are self-adhesive patented dual layer hydrogels that are recommended for attachment of the AP. This monitor has an 8-bit analog to digital converter, a sampling frequency of 10 Hz, and a memory of 4 Mb that allows recording of data in excess of 7 days. The AP provides output that can be downloaded to a computer in the form of daily and hourly activity, which is classified as time spent sitting/lying, standing, and stepping.

AP was selected because the studies that have examined the validity and reliability of AP are limited in the apparently healthy overweight/obese adults and healthy lean adolescents. Most of the previous studies have been in adult patients and the AP was used to measure the time spent in static activities (sitting/lying and standing) and dynamic activity (stepping and step cadence (steps.min^{-1})) (Dahele *et al.*, 2007; Godfrey *et al.*, 2006; Koulouri *et al.*, 2006; Godfrey *et al.*, 2008). AP was found to be reliable and valid in terms of assessing step number and cadence/ pace during walking (Ryan *et al.*, 2006). Some of the previous studies recommended that a validation study comparing direct physical activity estimated by AP with gold standard measurement is

required (Dahele *et al.*, 2007). In addition, AP provides assessment of general physical activity level in EE. The EE obtained from AP is based on the default values of (MET) (1.25 MET for sitting/lying, 1.4 MET for standing and 4 MET for stepping at a cadence of 120 steps per minute (brisk walking pace)) and EE calculated according to the formula (Ainsworth *et al.*, 2000):

$$EE\ (MET.h^{-1}) = (1.4 \times \mathbf{d}) + (4 - 1.4) \times (\mathbf{c}.120^{-1}) \times \mathbf{d}$$

c= cadence (steps per minutes), **d**= activity duration (hours)

This formula used to assess general physical activity EE might be suitable for adults. However, AP could not discriminate between complex physical activities such as non-step based physical activity (Dahele *et al.*, 2007). Few studies have examined the validity and reliability of AP in estimating EE in healthy adults especially in overweight/obese individuals during light and moderate physical activity including activities such as walking on the flat and uphill. Furthermore, the first study of this thesis is the first study to examine the validity and reliability of AP in estimating EE in lean adolescents during walking on the flat and on an inclined surface.

1.3.3 SenseWear PRO_2 Armband

The third selected type of movement sensing device is SenseWear PRO_2 (SW). The SW Armband is manufactured by BodyMedia Inc.^{TM}, USA. It has been developed for clinical and health research. Unlike to AG and AP, SW includes 2-axis accelerometer and has a number of sensors to gather health related information such as skin temperature, near body temperature, heat

flux, movement, and galvanic skin response in conjunction with body measurements such as sex, age, height, and weight to calculate energy expenditure (Mealey, 2008). This device is a physiological-based device and designed to be worn on the back of the right upper arm (on the triceps muscle) with touching the skin. In a previous study, Fruin and Rankin (2004) compared activity EE ($Kcal.min^{-1}$) assessed by SW in adults using InnerView^{TM} version 1.0 (BodyMedia, Inc.) with indirect calorimetry (IC) during walking on a treadmill on a flat surface and at a 5% uphill gradient. The results showed that SW significantly overestimated PAEE in lean adults (25.2$\pm$3.2 yrs) compared to indirect calorimetry during walking on a level treadmill by 38% at 80.5 $m.min^{-1}$ ($\sim$4.83$km.h^{-1}$) and by 14% at 107.3 $m.min^{-1}$ ($\sim$6.44$km.h^{-1}$) $P<0.02$, and underestimated PAEE during walking at 107.3 $m.min^{-1}$ on a 5% gradient by 22%, $p<0.01$ (Fruin and Rankin, 2004).

More previous studies have reported limitations with the use of SW in children and adolescents. The first study was investigated by Arvidsson and colleagues (2007). They found that SW with using software version 5.1 underestimated resting activities by 18.6% and light intensity game playing by 35.7% and during walking at speeds 1.9 mph, 2.5 mph and 3.1 mph SW (using version 5.1) underestimated activity EE by 0.8%, 8.6% and 9.7% respectively (Arvidsson *et al.*, 2007). In contrast, Dorminy *et al.* (2008) used the same device (SW) but with different software (version 4.2) to assess EE in children. They reported an overestimation of EE for resting activity as well as some other sedentary activities by 21.2% and 21.1% respectively. During walking at speeds ranging between 2.5 and 4.5 mph, the average error was

about 14.2% (Dorminy *et al.*, 2008). In a recent study, Calabro and colleagues (2009) used the same device (SW) with the most recent software version 6.1. They found that the new software version 6.1 provided an average of error in EE assessment of -20.7%, -4.0%, -4.9%, -0.9%, +0.6% and +3.5% for resting, colouring, computer games, walking on a treadmill at speeds 2, 2.5 and 3 mph, respectively, in 9.4(±1.3) year old children. They concluded that the results demonstrate that the most recent version 6.1 provides more accuracy in assessing some of the typical physical activity in young children (Calabro *et al.*, 2009).

Although SW is provided with different components to assess EE such as integrating its heat flux and accelerometry values to accurately detect energy cost of physical activities it is clear that some problems exist despite a noticeable improvement observed in the SW's software between the more recent version 4.2, 5.1 and 6.1. The SW device with the new version 6.1 was able to eliminate some of the problems of EE estimation. However, there are very few studies investigating the validity and reliability of SW with the most recent version of software (6.1) especially in overweight/obese adults and in lean adolescents during some of the most common physical activities such as walking at different speeds. Moreover, there are no studies examining the validity and reliability of SW using this most recent software during walking on an incline surface. Thus, more research is needed in overweight/obese individuals and adolescents in order to provide some information to help to improve the algorithms of the new coming software versions so that PAEE can be assessed precisely.

1.4 Physical activity assessments using objective measures

Accurate assessment of the physical activity intensities in terms of sedentary, light, moderate, vigorous, and very vigorous activities, may help us to understand the relationship between physical activity status, adiposity status and health status in adults and adolescents. Therefore, the first step in understanding these relationships is to assess habitual physical activity precisely by using objective measures such as accelerometry and heart rate monitoring and applying the most appropriate methods and procedures for interpretation and analysis of the data. The next step is to compare the assessed physical activity to the most recent recommendations and guidelines for health and wellbeing.

1.4.1 Physical activity assessments in adults

There are several studies that have indicated that physically inactive adults are more likely to have suffer from chronic diseases such as cardiovascular disease (Panagiotakos *et al.*, 2003; Fang *et al.*, 2003), osteoporosis (Kai *et al.*, 2003) and diabetes (Kriska *et al.*, 2003; Hu *et al.*, 2003). Today, physical activity assessment has become more accurate with the use of the newly developed objective measures such as accelerometers and heart rate monitors. In the last decade, the number of studies that have used AG to assess habitual physical activity has increased (Hendelman *et al.*, 2000; Kwak *et al.*, 2007; Davis *et al.*, 2006). Ward *et al.* (2005) investigated the best practices and research recommendations in order to improve the accuracy of AG. The new

generation of the AG was found to be a valid and reliable device in assessing physical activity in terms of intensity and time spent in the activity (duration) as well as providing more data such as step counts (Caspersen *et al.*, 1985; Ekelund *et al.*, 2006; Johansson *et al.*, 2006; Mâsse *et al.*, 2005). However, few studies have used the improved and valid new generation of AG GT1M to assess the habitual physical activity of the apparently healthy lean versus overweight/obese adults.

On the other hand, the recommended guidelines of physical activity for adults may vary according to the health aim. Since 1995, the physical activity recommendations for adults were changed for better public health promotion. In 1995, the Centres for Disease Control (CDC) and the American College of Sports and Medicine (ACSM) established physical activity recommendations of 30 minutes or more of moderate intensity physical activity per day, preferably all days of the week for adults for the purpose of health benefits (Pate *et al.*, 1995).

In 2007, ACSM and American Heart Association (AHA) updated the physical activity and exercise recommendations for healthy adults (18-65yr) to promote and maintain health (Haskell *et al.*, 2007). They recommended moderate intensity aerobic physical activity for a minimum of 30 minutes on five days per week, or vigorous intensity of aerobic physical activity for a minimum of 20 minutes on three days per week. A combination of moderate and vigorous intensity of aerobic physical activity can be performed to meet this recommendation. Recently, the Department of Health and Human Services

(DHHS, 2008) issued physical activity guidelines for American adults (U.S. Department of Health and Human Services, 2008).

The DHHS published recommendations of 30 minutes or more of moderate-intensity physical activity for healthy adults on most days of the week ($\geq$5d.wk^{-1}) for health and to reduce the risk of chronic disease. Scotland Health Survey (SHeS) and Health Education Population Survey (HEPS) both published similar recommendations for healthy adults. They recommended at least 30 minutes of at least moderate intensity activity on most days per week ($\geq$5d.wk^{-1}) for health benefits (Catto *et al.*, 2009).

On the other hand, for the aim of weight loss and for additional health benefits, the DHHS recommended at least 300 minutes (5 hours) of moderate-intensity per week, or 150 minutes (2.5 hours) of vigorous-intensity activity per week, or an equivalent combination of moderate and vigorous intensity aerobic physical activity (U.S. Department of Health and Human Services, 2008).

1.4.2 Physical activity assessments in adolescents

Today, there is much evidence to indicate that an adequate physical activity programme should start at an early age such as in childhood (Morris et al., 1953). The benefit of being active at an early age can carry over into adulthood as active children are likely to be more active when they become adults (Strong et al., 2005; Currie et al., 2004). In a recent study, Hallal and colleagues (2006) concluded that physical activity in early age such as childhood or adolescence may help to improve healthy lifestyle choices in adulthood which may reduce

the risk of developing some of the chronic diseases such as cardiovascular and metabolic diseases (Hallal et al., 2006). Regardless of adiposity, adolescents with higher cardiorespiratory fitness have been found to be at lower risk of cardiovascular disease (Martins et al., 2010). Furthermore, other studies have found that some of the chronic diseases such as cardiovascular and metabolic diseases that appear in adulthood are developed in childhood (Parsons et al., 1999). However, the health benefits of physical activity in adolescents are not as widely investigated as in adults (Currie et al., 2004). On the other hand, Strong and colleagues (2005) reviewed 850 articles in a systemic review to review the effects of habitual physical activity on health and to develop evidence-based recommendations for physical activity in youth. They concluded that school-age youth should engage in at least 60 minutes of moderate to vigorous physical activity in order to achieve the desired health and behavioural beneficial outcomes (Strong *et al*., 2005). Therefore, this evidence raises the importance of assessing physical activity in adolescents accurately in order to improve the recommended physical activity guidelines for adolescents for better health benefits. However, physical activity assessment for children and adolescents is not easy and is still a big challenge (Armstrong and Welsman, 2006).

An accurate and valid measure with appropriate methods is important to assess physical activity levels for adolescents. Objective measures such as accelerometry have been found to be valid and accurate enough for habitual physical activity assessment in adolescents (Puyau *et al.*, 2002; de Vries *et al.*, 2006). Particularly, AG is one of the researched accelerometers in a

systematiic review study that has been found to be reproducible, valid, and feasible for use in children and adolescents (2-18yr) (de Vries *et al.*, 2006). For the reason of improving public health policy, accurate assessment of physical activity for adolescents has become a priority for health promotion (Riddoch *et al.*, 2004). Parallel to the recent studies in other European countries (Riddoch *et al.*, 2004), this thesis will contribute with other studies to provide accurate information about the habitual physical activity status for a sample of adolescents in Central Scotland using valid and reliable objective measures (AG and HR monitoring). Thus, an accurate assessment of physical activity in Scottish adolescents in relation to health risk factors such as cardiovascular and metabolic diseases is needed (Steene-Johannessen *et al.*, 2010; Watts *et al.*, 2005).

1.5 Physical activity and cardiovascular and metabolic disease risk markers

1.5.1 Cardiovascular and metabolic disease

Cardiovascular disease is defined as coronary heart disease but also including stroke, peripheral vascular disease, and heart failure (Wallis *et al.*, 2000). Coronary artery disease, heart attack, hypertension and atherosclerosis are manifestations of cardiovascular disease. Physical activity has been found to be a primary prevention factor for coronary heart disease (CHD) as physically active individuals are at lower risk for CHD than less active persons (Eichner, 1983; Oberman, 1985; Paffenbarger and Hyde 1984). Metabolic disease is defined as one or more of the following conditions: non–insulin-dependent

diabetes mellitus, type II; treated hypertension; obesity; high insulin; and unhealthy lipid profile in the blood (Boon *et al.*, 2001).

The influence of physical activity status or exercise participation on the key risk markers of cardiovascular and metabolic diseases has been investigated in a number of studies in adults (Jakicic *et al.*, 1993; Brown *et al.*, 2007; Monzillo *et al.*, 2003; Monzillo *et al.*, 2003; Laaksonen *et al.*, 2002), and in adolescents (Hulver *et al.*, 2003; Kelly *et al.*, 2007; Rizzo *et al.*, 2008; Ischlander *et al.*, 2007). However, the association with physical activity intensity per se on the cardiovascular and metabolic disease risk markers is not fully clear and more studies in adults (Engeli *et al.*, 2003; Monzillo *et al.*, 2003) and adolescents (Jebb and Moore, 1999; Jakicic *et al.*, 1993) are suggested. Thorseng and colleagues (2009) concluded that the role of physical activity on cardiovascular and metabolic disease risk factors is not fully understood and more investigations are deserved.

On the other hand, studies in adolescents do not agree about the association between physical activity and some of the cardiovascular and metabolic disease risk markers (Hulver *et al.*, 2003; Kelly *et al.*, 2007, Blüher *et al.*, 2006; Ischlander *et al.*, 2007).

1.5.2 Association between physical activity status and cardiovascular and metabolic disease risk markers in adults.

Physical inactivity and body composition have been found to play key roles in the development of some of the cardiovascular and metabolic disease risk

markers independently (Jakicic *et al.*, 1993; Cho *et al.*, 2009; Rokling-Andersen *et al.*, 2007; Kondo *et al.*, 2006). Obesity as a health problem has been found to be associated with the classical and the newer cardiovascular and metabolic disease risk markers. For example, a high percentage of body fat was found to be associated with high blood pressure, elevated blood lipids (high total cholesterol, LDL and low HDL) (Engeli *et al.*, 2003; Cho *et al.*, 2009; Kannel *et al.*, 1967), and was associated with some of the newer cardiovascular disease risk markers such as leptin and some of the inflammatory risk markers such as C-reactive protein (CRP), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Hong *et al.*, 1996; Ridker *et al.*, 2002; Greenberg and Obin, 2006; Maffei *et al.*, 1995). However, as mentioned previously, there are many studies which have demonstrated the health benefits of performing a sufficient amount of physical activity for adults even in overweight/obese adults (19,Farrel *et al.*, 2007;,Blair and Brodney, 1999). In a comprehensive literature review, active obese adults were found to be at lower morbidity and mortality than sedentary lean adults (Blair and Brodney, 1999) suggesting that activity status has a bigger impact than adiposity on risk of disease. However, body fat stores themselves are responsible for production of many of the new cytokine risk markers of disease; therefore, the association of physical activity to some of the traditional and newer cardiovascular metabolic disease risk markers are not fully understood.

1.5.2.1 Association between physical activity and some of the traditional cardiovascular metabolic disease risk markers

The effect of physical training on the cardiovascular disease risk markers has been examined in a number of previous studies (Rokling-Andersen *et al.*, 2007, Blair *et al.*, 1984; Polak *et al.*, 2006). In a recent study, Polak and colleagues (2006) investigated the association of exercise training (12 weeks) at moderate intensity with plasma levels of some adipokines and blood lipid markers for overweight adults. Although the training programme significantly reduced body weight, BMI and percentage of body fat (all $P<0.01$), there were no significant change in total cholesterol, HDL, triglyceride or fasting glucose concentrations (Polak *et al.*, 2006). According to the National cholesterol education programme (NCEP) criteria of the metabolic syndrome (National cholesterol education programme, 2001), the participants in Polak's study were within the risk zone even after an exercise training programme (Polak *et al.*, 2006). They referred their results to the type and duration of the training session as well as the sex and degree of obesity of the participants. Therefore, they concluded that the influence of physical activity as a health factor may appear only in long term lifestyle changes. However, there is some evidence showed that exercise intensity may be important as the effect of exercise training on blood lipid for obese women – but not for the lean women has been associated with high physical exercise intensity in one study (Rokling-Andersen *et al.*, 2007). In a review of literature by Wiliams (2001), it was shown that runners who were doing high intensity exercise had changes in HDL that were strongly dependent upon loss of weight. As discussed earlier, cardiovascular disease risk markers

increase in adults with increases in body fat (Williams, 2001). Thus, blood lipid concentrations as classical cardiovascular disease risk marker may be affected more by reducing fat than by physical activity especially in inactive lean adults, unless the activity intensity is high. Furthermore, exercise intensity may be the key factor in the positive changes in some of these risk markers at least in obese adults (Rokling-Andersen *et al.*, 2007).

Free fatty acids such as Omega 3 (n-3) and omega 6 (n-6) ratio have been proposed as cardiovascular risk markers (Bell *et al.*, 2010). More n-3 and less n-6 are desirable for cardiovascular health indication and the balance of n-3 and n-6 is an important index for cardiovascular diseases (Thorseng *et al.*, 2009; Lands, 2003). Furthermore, n-3 has been found as a protector against insulin resistance (Thorseng *et al.*, 2009). Daily food intake such as fish oil is the main factor for increasing n-3 and reducing n-6 levels (Marangoni *et al.*, 2007). However, there are some other factors such as obesity and physical activity status that may play a role in these risk marker levels (Thorseng *et al.*, 2009; Mozaffarian, 2008). Exercise intensity can promote the utility of fatty acids as energy substrates, the mobilization of fatty acid reserves of the adipose tissue, and in the lipid transport between different tissues (Holloszy and Kohrt, 1996; Nikolaidis and Mougios, 2004). Thus, physical activity status may contribute as a health factor along with free fatty acid balance for better prediction of cardiovascular diseases (Mozaffarian, 2008; Deckelbaum *et al.*, 2008).

On the other hand, fasting insulin and glucose as classic metabolic disease risk markers have been found to be significantly associated with physical activity

with or without obesity (Kelley and Goodpaster, 1999). However, Ross and colleagues (2004) found that in obese women, exercise with or without weight loss had no effect on fasting insulin levels compared to a control group. However, the absence of the physical activity or exercise influence on fasting insulin probably related to the normal level of fasting insulin before exercise (Choo *et al.*, 2010). Hawley and Lessard (2008) demonstrated that a beneficial effect of exercise training on a constant increase in insulin sensitivity was observed in skeletal muscle from obese adults. Furthermore, the influence of physical activity could be related to the changes in other factors such as some of the newer risk markers (e.g. leptin) that play a role in fasting insulin and glucose levels (Franks *et al.*, 2007). A previous study demonstrated that leptin acts as an insulin sensitizer when leptin concentrations are at low or normal levels and may contribute to insulin resistance when leptin is constantly elevated (Franks *et al.*, 2007).

However, the association between low physical activity status versus high activity status and classical cardiovascular and metabolic risk markers in apparently healthy adults requires further investigation.

1.5.2.2 Association of physical activity and some of the newer cardiovascular metabolic disease risk markers

Adiponectin, leptin, high sensitivity C-reactive protein (hsCRP), IL-6 and TNF-α are known as newer cardiovascular and metabolic risk markers and have been found to be affected by some factors such obesity, physical activity and

exercise intensity (Rokling-Andersen *et al.*, 2007,Kondo *et al.*, 2006, Mattusch *et al.*, 2000; Christensen *et al.*, 1998).

Adiponectin

Adiponectin is known as a hormone induced by adipose tissue and found to enhance insulin sensitivity and increase free fatty acid (FFA) oxidation in several tissues including muscle tissue (Fruebis *et al.*, 2001; Yamauchi *et al.*, 2001; Scherer *et al.*, 1995). It has been demonstrated that low adiponectin concentration is correlated with insulin resistance and hyperinsulinemia as well as coronary artery disease (Hotta *et al.*, 2000; Hotta *et al.*, 2001). Adiponectin has been found as a better predictor of endothelial function of the coronary artery than other blood markers such as homeostasis model assessment-Insulin resistance (HOMA-IR), immunoreactive insulin and triglycerides (Okui *et al.*, 2008).

Physical activity and adiponectin

Some studies have failed to find a significant association between physical training and plasma adiponectin. In a recent study, exercise training programme ($30min.d^{-1}$ at 50% of VO_{2max}, $5d.wk^{-1}$, for 3 months) did not influence adiponectin concentrations in obese adults (Polak *et al.*, 2006). However, long-term endurance physical exercise at moderate intensity may increase adiponectin level. Kondo and colleagues (2006) investigated the effect of exercise on circulating adipokines including adiponectin in obese young women (Kondo *et al.*, 2006). They examined the influence of 7 months of

endurance exercise at 60-70% of $HR_{max\ reserve}$ for about 30 minutes per week on adiponectin concentration in obese and lean females. The results showed significant increase in adiponectin in the obese females ($P<0.01$), but not significant in lean females (Rokling-Andersen *et al.*, 2007). Thus, the association of physical activity status and plasma adiponectin concentration as a health risk predictor in healthy adults is not fully clear. Therefore, more studies may be needed to clarify the relationship of the physical activity status and adiposity with circulating adiponectin in apparently healthy adults.

Leptin

Leptin is a hormone secreted mainly from white adipose tissue (Berggren *et al.*, 2005). Leptin is recognised as one of the newly discovered cardiovascular and metabolic risk markers (Wannamethee *et al.*, 2007). Furthermore, leptin is thought to influence energy intake by a direct effect on the hypothalamus (Maffei *et al.*, 1995).

Physical activity and leptin

The effect of exercise duration and physical activity intensity on plasma leptin has been examined in several studies (Monzillo *et al.*, 2003; Polak *et al.*, 2006; Jürimäea *et al.*, 2009; Berggren *et al.*, 2005; Wannamethee *et al.*, 2007). Duration and intensity of physical activity training may play a role in the plasma leptin concentrations. For example, long-duration of exercise training (Perusse *et al.*, 1997) and sufficient high intensity of physical activity (Elias *et al.*, 2000) have been shown to decrease the circulating leptin concentrations in adults

independently. In contrast, short-term exercise training did not influence fasting plasma leptin concentration (Houmard *et al.*, 2000). Therefore, the influence of physical activity or exercise training seems to be unclear but may be related to longer term habitual activity. Although the influence of long-term exercise training has been related to the reduction of body weight (Christensen *et al.*, 1998), other studies have demonstrated that the effect of the long-term exercise training on leptin concentration was independent of weight reduction (Kondo *et al.*, 2006; Hickey *et al.*, 1997). Yet, it is not fully clear whether or not a low physically active lifestyle (e.g. MVPA <150mins/w) –but not sedentary- is enough to influence plasma leptin concentrations positively, especially in apparently lean healthy adults. Further research is suggested to investigate whether or not physical activity status is related to positive differences in plasma leptin concentrations.

High sensitive C-reactive protein (hsCRP)

The hsCRP (or CRP) is synthesized and released by different cells in response to microbial infection, tissue injury and immunomodulatory stimuli (Haider *et al.*, 2006). The hsCRP is an independent predictor of cardiovascular disease and an important marker of inflammation (Ridker *et al.*, 2002, Danesh *et al.*, 2004; Lindmark *et al.*, 2001).

Physical activity and hsCPR

In a recent study, Andersson *et al.* (2009) examined the effects of heavy endurance physical exercise on inflammatory markers in non-athlete lean

adults. They found that hsCRP was decreased significantly during the recovery period following a high intensity physical exercise programme (Andersson *et al.*, 2009). High intensity of physical activity may reduce the hsCRP concentration even in lean adults with normal hsCRP concentrations (Andersson *et al.*, 2009; Franks *et al.*, 2007). However, there are limited studies examining the association between low or moderate physical activity and the circulating hsCRP in healthy adults. The hsCRP as an independent predictor of cardiovascular disease as well as a key marker of inflammation (Ridker *et al.*, 2002; Danesh *et al.*, 2004; Lindmark *et al.*, 2001) deserves more focussed research to investigate the role of physical activity status on hsCRP concentrations in apparently healthy adults.

Interleukin (IL-6)

IL-6 is a multifunctional cytokine secreted by deferent cell types including immune cells, skeletal muscle, and adipose tissue. Elevated plasma IL-6 concentrations play a major role in predicting future myocardial infarction in healthy adults (Ridker *et al.*, 2000).

Physical activity and IL-6

Physical exercise has been found to be associated with plasma IL-6 concentrations (Ullum *et al.*, 1994; Ostrowski *et al.*, 1998; Drenth *et al.*, 1995). Ullum *et al.* (1994) found that plasma IL-6 elevated significantly in healthy moderate trained men during exercise cycling at 75%VO_{2max}. Similar results were observed by Ostrowski and colleagues (1998) in trained men. They

examined the effect of running at 75%VO_{2max} intensity on cytokines including plasma IL-6 in trained men. They found that plasma IL-6 increased after 30minutes of exercise and by 25-fold after 2.5 hours of exercise comparing to per exercise level. They also, observed significant elevation of plasma IL-6 even after 6 hours of exercise. However, plasma IL-6 decreased to baseline level (pre exercise) on the second day after the exercise test (Ostrowski *et al.*, 1998). They explained these findings as a result of muscle damage during exercise, which are supported by other studies (Bruunsgaard *et al.*, 1997; Lieber *et al.*, 1996)

On the other hand, an effect of short-term exercise training on plasma IL-6 is absent. Recent studies have failed to find significant differences in plasma IL-6 concentrations between pre and post exercise training programmes (Polak *et al.*, 2006; Fischer *et al.*, 2004). Polak and colleagues (2006) did not find significant change in plasma IL-6 in overweight women after 3 months of aerobic exercise at 50%VO_{2max} even after significant increased in VO_{2max} and significant reduction in fat mass. However, some studies have found that IL-6 secreted from subcutaneous adipose tissue was positively correlated with BMI and fat mass (Kern *et al.*, 2001), and a number of studies have also demonstrated that plasma IL-6 concentration decreased significantly in obese adults after body weight reduction (Monzillo *et al.*, 2003; Bastard *et al.*, 2000; Ziccardi *et al.*, 2002).

The influence of physical activity status on IL-6 is still unclear and the effect of physical activity status seems to be not independent of other factors such as weight reduction (Monzillo *et al.*, 2003). The association between physical

activity per se and plasma IL-6 concentration is therefore not fully understood. Polak and colleagues (2006) have suggested that more investigations about the effect of physical activity status on plasma IL-6 concentrations in healthy adults are required.

Tumour necrosis factor-alpha (TNF-α)

TNF-α is a proinflammatory cytokine consider to be a critical mediator of insulin resistance (Hotamisligil *et al.*, 1993). However, the role of TNF-α in the development of insulin resistance in humans is unclear and more studies are required (Dyck *et al.*, 2006).

Physical activity and TNF-α

Plasma TNF-α as a proinflammatory marker has been investigated under physical exercise interventions in some studies (Polak *et al.*, 2006, Sloan *et al.*, 2007; Andersson *et al.*, 2009). Findings in some research did not observe any association between moderate exercise training and TNF-α concentration (Polak *et al.*, 2006; Sloan *et al.*, 2007). Similar to the results found in IL-6, TNF-α concentration did not change in overweight women after short-term aerobic exercise training at 50% VO_{2max} even after a significant reduction in body fat (Polak *et al.*, 2006). In contrast, TNF-α concentration was found to be affected by high intensity of exercise. Sloan *et al.* (2007) demonstrated that 3 months of high exercise intensity caused a significant reduction in inducible TNF-α synthesis in whole blood in sedentary adults. However, the influence of a high intensity of exercise training probably was temporary because Andersson and

colleagues (2009) observed that heavy exercise training increased TNF-α significantly in fit men -but not athletic- in the first and second weeks of the training programme. However, they found that the TNF-α concentrations returned to the baseline level during the recovery period time of the sixth and eighth weeks (Andersson *et al.*, 2009). Thus, the role of physical activity on the circulated TNF-α as a proinflammatory marker is possibly related to indirect mechanisms. Further investigations are needed in order to fully understand the role of physical activity status on the circulating TNF-α concentration in healthy adults including lean and overweight and obese individuals.

1.5.3 Physical activity and cardiovascular and metabolic disease risk markers in adolescents

The studies that have investigated the association between physical activity status and some of the key traditional and newer cardiovascular and metabolic disease risk markers in adolescents are limited especially in healthy adolescents. Furthermore, the results of previous studies have not always agreed especially in regard to the recently discovered key cytokines (Kelly *et al.*, 2007; Ischlander *et al.*, 2007, Moore *et al.*, 2008).

1.5.3.1 Association between physical activity and some of the traditional cardiovascular metabolic disease risk markers in adolescents

There are number of studies that have investigated the effect of physical activity on some of the classical cardiovascular and metabolic disease risk

markers in adolescents (Hallal *et al.*, 2006, Medina-Urrutia *et al.*, 2008; Huang *et al.*, 2007; Andersen *et al.*, 2003; Steinberger *et al.*, 2009; Andersen *et al.*, 2006). Although some of these studies found associations between physical activity status and some of the key risk markers (Brage *et al.*, 2004), others demonstrated that there is no relationship between physical activity and some of these key risk markers, such as total cholesterol and blood pressure (Hallal *et al.*, 2006). For example, Brage and colleagues (2004) investigated the association between objectively measured fitness and the metabolic syndrome in Danish children (age 9.6yr). They concluded that physical activity and fitness were inversely associated with the metabolic risk markers. In contrast, a systematic review concluded that physical activity status in adolescents had no consistent effect on lipid concentrations, fasting glucose or blood pressure (Hallal *et al.*, 2006). Therefore, the influence of physical activity status and the traditional cardiovascular and metabolic disease risk markers is not fully understood for adolescents. Therefore, more research is needed to explore the relationship between objectively measured physical activity status and the traditional cardiovascular and metabolic disease risk markers in healthy adolescents.

1.5.3.2 Association between physical activity and some of the newer cardiovascular metabolic disease risk markers in adolescents

Several studies have investigated the effect of physical activity or exercise training on some of the newer cardiovascular and metabolic disease risk markers such as cytokines in adolescents (Kelly *et al.*, 2007; Isasi *et al.*, 2003;

Moore *et al.*, 2008). However, the association between the physical activity status itself and these key risk markers is not clear because the results of the previous studies have not fully agreed (Kelly *et al.*, 2007; Ischlander *et al.*, 2007; Moore *et al.*, 2008). For instance, Rubin *et al.* (2008) examined the association between vigorous physical activity and some of the key cytokines in adolescents using a questionnaire. They found that there were no significant differences between adolescents with high versus low self reported vigorous physical activity in all examined cytokines including adiponectin (Rubin *et al.*, 2008). Although some other studies found that there is a significant association between physical activity and some of these key risk markers, the results were explained as a result of body fat reduction (Kelly *et al.*, 2007). Kelly and colleagues (2007) investigated the effect of the physical exercise intervention on some of the key cytokines in overweight children (11±0.7yr). Particularly, exercise training itself did not improve the adipokine profile including adiponectin, IL-6, TNF-α and hsCRP in the absence of weight loss (Kelly *et al.*, 2007). In contrast, some other studies showed that physical activity plays a role in positive changes in some of the key cytokines concentration (Blüher *et al.*, 2006,Ischlander *et al.*, 2007). Bluher *et al.* (2006) and Ischlander *et al.* (2007) demonstrated that the effect of physical activity, or exercise training, on some of the cytokines were positive and independent of the change in body fat. Most of the previous studies were in children (below 12yrs) and few of these studies use objective measures for physical activity assessment such as accelerometry or heart rate monitoring. Further research seems necessary in order to help understand the relationship between physical activity status and key adipokines in adolescents.

1.6 Aims of the studies in this thesis

The contribution of this thesis is to evaluate some of the objective physical activity measures and the habitual physical activity in relation to cardiovascular and metabolic disease risk markers for adults and adolescents. Thus, five separate studies are conducted and the aims of these studies are:

1.6.1 Aims of the first study

To evaluate the validity and reliability of the new generation of three of the most popular movement sensing devices in assessing activity EE compared to indirect calorimetry under the same protocol for people including lean and overweight/obese adults (18-55yr) and adolescents (12-17yr) in order to contribute in assessing physical activity energy expenditure more precisely.

1.6.2 Aims of the second study

The aim of the second study in this thesis was to assess the free living habitual physical activity of apparently healthy lean and overweight/obese adults (18-55yr) using a new generation of AG (GT1M) with consideration of the recommended best practice for using AG as well as using a HR monitor and to compare the habitual physical activity between groups (lean vs. overweight/obese).

The second aim was to assess the agreement between the physical activity levels of the adult participants (lean and overweight/obese) with the national and international physical activity recommended guidelines for healthy adults.

1.6.3 Aims of the third study

Following objectively measuring free living physical activity in adults the main aims of the third study were:

To investigate the association between key metabolic and cardiovascular risk markers with body composition and habitual physical activity status in apparently healthy adults.

To investigate whether or not there is health benefits (reductions in risk markers) of physical activity below and above the recommended physical activity guidelines for adults regardless of adiposity status.

1.6.4 Aims of the fourth study

The main aim of the fourth study was to assess the free living habitual physical activity of apparently healthy male and females adolescents (12-17yrs) using an Actigraph accelerometer (GT1M) and HR monitor.

The second aim was to compare the physical activity data of the adolescents in the present study to similar data of adolescents in different countries.

1.6.5 Aims of the fifth study

Following objectively measuring physical activity in adolescents, the main aims of fifth study were to investigate some of the important cardiovascular and metabolic disease risk markers in relation to physical activity status in apparently healthy lean adolescents.

The second aim was to compare the results of the present study to the results from similar studies in adolescents.

Chapter 2- Reliability and validity of accelerometer devices in lean and overweight adults: effects of walking speed and gradient.

2.1 Introduction:

Given the clear association between habitual physical activity and health it is becoming increasingly important to be able to monitor and assess activity status in the population. Accurate assessment of physical activity may help to explain and prevent the dramatic increase in overweight and obesity prevalence observed among children, adolescents and adults (Petersen *et al.*, 2004; Pate *et al.*, 2002). Therefore, focused attention on improved assessment of physical activity may be required. Recently, there have been many types of objective instruments developed to assess habitual physical activity status as well as activity energy expenditure (EE) (Schmidt *et al.*, 2003). However, not all of these instruments are appropriate devices for measuring EE, and many have not been validated for use in studies on apparently healthy adolescents (Vogels *et al.*, 2007), nor comparing apparently healthy obese/overweight with lean adults. Evaluation of the validity and reliability of some available accelerometer devices that are routinely used to assess EE and physical activity (PA) may need to be examined.

Since 1997, studies examining the calibration, validity, reliability, and development aspects of different types of accelerometer devices have been increased (Strath *et al.*, 2003). One of the conclusions from the consensus meeting that was held in the Cooper Institute in October, 1999 was that

objective motion sensors were not practical for large-scale studies because of high cost, uncertain reliability, and difficulties in the interpretation of data (Vogels *et al.*, 2007).

Some accelerometer devices provide an indication of overall movement but a fundamental research challenge has been to determine how accelerometer counts equate to more meaningful indicators such as activity EE or time spent in light, moderate and vigorous activities (Troiano RP. 2005). However, some of these challenges exist because accelerometer devices are based on biomechanical principles while energy expenditure and oxygen consumption are biological measures. Particularly, growth and maturation of children and adolescents lead to perturbation in some studies (Cole *et al.*, 2000). For example, one of the challenges that has been met in different studies was that the equations of these devices are based on locomotor patterns. In turn, the devices tended to overestimate EE of low-level activities, while underestimating EE of relatively high intensity exercise (king *et al.*, 2004; Schmidt *et al.*, 2003). Current calibration research concluded that continued studies on validity, reliability and calibration of different types of accelerometers are needed (Brage *et al.*, 2003; Chen and Bassett, 2005; Freedson *et al.*, 2005; Jakicic *et al.*, 1999). There are several studies that have assessed physical activity status of adolescents and adults using accelerometry devices (Brage *et al.*, 2003; Freedson *et al.*, 2005; Troiano RP. 2005; Liden *et al.*, 2001; Crouter *et al.*, 2006b). However, few of these studies have examined the validity and reliability of the new generation of accelerometer devices in assessing physical activity EE in adolescents and adults (Brage *et al.*, 2003; Petersen *et al.*, 2004).

ActiGraph model GT1M (AG), ActivPAL (AP) and SenseWear PRO$_2$ (SW) are examples of the new generations of some of the movement sensing devices that have been recently developed in order to assess physical activity and EE more precisely.

The contribution of the present study is firstly to evaluate the validity and reliability of these devices in assessing EE compared with indirect calorimetry during different walking speeds on the flat and during uphill walking, where EE is greater but step counts are the same. Secondly, this study aims to evaluate the validity and reliability of these devices in overweight/obese adults and normal weight adolescents. Walking at different speeds was selected because it is a common physical activity in both adults and adolescents during their free-living activity.

2.1.1 Aims of the study:

1. Assess the reliability of the new generation of the three accelerometers (AG, AP, SW) in estimating EE during walking by comparing EE obtained from these devices during walking on a treadmill at 3 different speeds on the flat and at 5% gradient in two laboratory sessions conducted one week apart in a group of adults and adolescents.
2. Assess the validity of these three accelerometers (AG, AP, SW) in assessing EE during walking by comparing EE obtained from these devices to the EE measured by indirect calorimetry during walking on a

treadmill at 3 different speeds on the flat and at 5% incline in adults and adolescents.

2.2 Methodology

2.2.1 Subjects

A total of 90 participants were recruited to take part in this study after successful approval for this study had been obtained from the research ethics committee at the University of Stirling and from the research ethics committee of Forth Valley and Fife NHS Trust, as well as a support letter from Children's Services division at Stirling Council. Participants were divided into two groups: adults and adolescents. 61 overweight/obese and lean adults (males n= 28, females n= 33) aged 18 -55 years and 29 lean adolescents (males n=15, females n=14) aged 12-17 years old completed their participation in this study. Physical characteristics of the participants are indicated in Table 2.1.

Table 2.1 Physical characteristics (mean ±SD) of the adult and adolescent participants

Physical characteristics	Adults		Adolescents
	Lean	Overweight/Obese	Lean
	n= 30	n= 31	n= 29
Age (yrs)	34.9 ±10.5	39.0 ±7.9	14.4 ±1.7
Mass (Kg)	65.5 ±8.6*	86.2 ±14.6*	56.1 ±9.3
Height (cm)	169.2 ±9.4	170.0 ±8.4	167.1 ±10.3
BMI ($Kg.m^{-2}$)	22.6 ±1.3*	29.8 ±4.1*	20.2(<75th) ±2.8
Body Fat%	21.7 ±7.2*	35.7 ±12.4*	18.4 ±7.8
Fat Free Mass (Kg)	51.0 ±9.4	54.2 ±11.6	45.8 ±8.9
Mean arterial pressure (MAP)	88.0±10.1	95.0±9.8	84.7±9.5
Resting heart rate	67.3 ±8.8	68.8 ±11.9	65 ±10.5
Predicted VO_{2max} ($ml.kg^{-1}.min^{-1}$)	47.31 ±10.70	32.80 ±4.86**	51.94 ±12.35

* Significant differences between lean and overweight adults at $P< 0.05$

** Significant differences between lean and overweight adults at $P< 0.000$

All participants were recruited from the public people in Stirlingshire council area. Adults were mainly recruited from Stirling city including University of Stirling students, and adolescents were recruited mainly from the Stirling and urban area high schools (8 high schools). All participants were informed about the study by an invitation letter, poster and/or leaflet. Subsequently, interested participants who were interested to take part in the study contacted the principle researcher via email, letter or telephone. Then they were provided with an information sheet describing the study in detail. If they decided to take part in the study, an initial briefing visit (first visit) was arranged with them to explain all parts and stages of the study. The second and third visits to the laboratory for treadmill testing were arranged one or two weeks apart and at the same

time of day. In the case of adolescents, parent(s)/legal guardian were also asked to come with their child and present with them for all visits. At least two adults were with adolescents participants in the laboratory during all sessions. Maximum 3 participants were arranged per day.

2.2.2 Study design:

In the initial briefing visit, participants attended the lab for familiarisation and baseline screening and body measurements. During this visit, the study was explained in detail and all questions about participation were answered. If the participant was still interested to take part he/she was asked to complete and sign a physical activity readiness questionnaire (PAR-Q) (revised by ACSM, 2002) and if eligible to enter the study they were asked to sign a consent sheet. All adolescents and their parent(s)/legal guardian were informed that they should both read and answer the PAR-Q and then both of them should sign the consent in the same form. After that, body measurements were taken including resting heart rate, blood pressure, body mass, height, and body composition. Body fat was assessed using a bioimpedance analysis method which has been found to be a valid and reliable instrument (Nuñez et al. 1997, Utter et al. 1999). The body mass index (BMI) (body mass(Kg) x (height(m^2) $^{-1}$) was calculated for adults and percentile for adolescents was obtained using BMI reference curves chart for UK children (UK90) (Cole *et al.*, 1995). Details of the instrumentations used for this study are found in Table 2-2.

Table 2-2 Name and model of instruments used in the present study

Measurements	Name and details of the instrument
Indirect calorimetry	Sensormedics Vmax 29, Holland
Impedance analysis (Body Fat %)	TANITA, Model: TBF-105, TANITA UK Ltd., Made in USA
Blood pressure & resting HR	OMRON, Digital Blood Pressure Monitor OMRON Electronics Ltd, Model: HEM-405C
Body mass	John White & Son, (Weighing Machines) Ltd., Made in Scotland
Height	Holtain Ltd., Made in Britain

All instruments and equipment to be used in visits 2 & 3 were fully described. Each participant was given enough time to become familiar with the environment of the laboratory including walking on the treadmill and breathing through the mouth using a mouth piece with the nose closed by a nose clip. It was hoped that this session would help to familiarize the participants with the new environment so that they would become familiar with the laboratory and its equipment. The second visit took place one week later. During the second and third visits which were one or two weeks apart, each participant was asked to attend the laboratory and to wear three types of accelerometers as well as heart rate monitor. Figure 2.1 illustrates how these devices were worn on body.

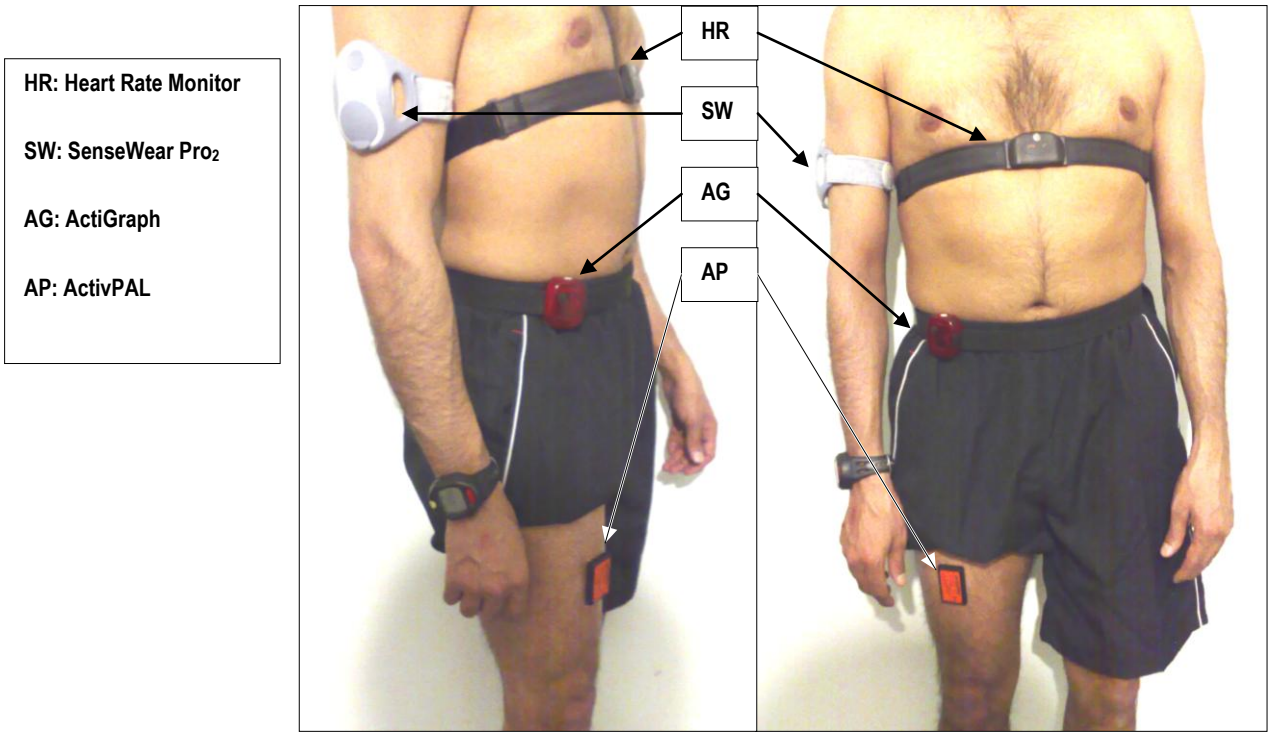


Figure 2.1 Heart rate monitor the three accelerometer devices attached on the body before starting the exercise test on the treadmill.

Expired gas analysis for determination of oxygen cost (VO_2) was prepared and calibrated with known gas volumes and gas concentration prior to each test for the participant. When the participant was ready, he/she was asked to have a seat on a chair placed on the treadmill and the investigator explained all stages of the test. Then a mouth piece and nose clip was fitted. The participant was then asked to remain relaxed on a chair for 5 minutes with the purpose of assessing baseline seated metabolic rate (obtained from minutes 4 and 5 of seated rest). Thereafter, they were asked to perform walking exercise on the treadmill at three different speeds (i.e. slow walking 3km/h, normal walking 4.5km/h and brisk walking 6km/h) in two different phases. In the first phase,

they performed 5 minutes for each speed on the flat (0% gradient). Then they were given 10 minutes seated rest on a chair for recovery. Thereafter, they performed 5 minutes for each speed on an incline (5% gradient). The experimental protocol for these two laboratory visits (exercise test) is illustrated in Diagram 2.1.

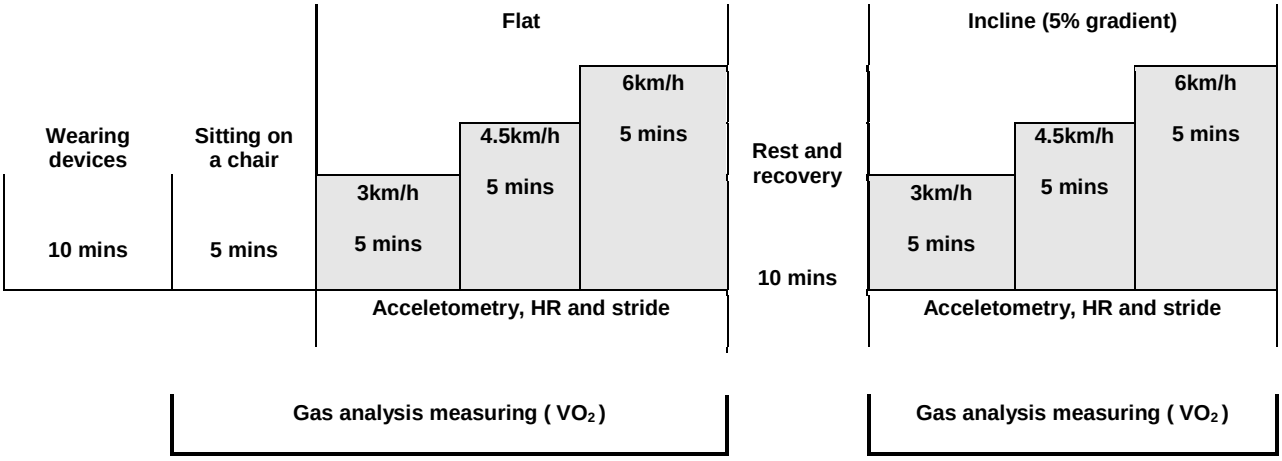


Diagram 2.1 Experiment protocol of the 2nd and 3rd laboratory visits

Oxygen cost ($VO_{2,}$) and energy expenditure ($kcals.min^{-1}$) were measured by indirect calorimetry gas analysis using Sensormedics Vmax29, Holland. Heart rate was measured continuously by heart rate monitor using Polar S625X^{TM} and averaged over the last 2 minutes of each stage. Three types of accelerometers were used to determine energy expenditure ($kcals.min^{-1}$). The accelerometers used in this study illustrated in Figure 2-2.

Figure 2.2 The three types of instruments that were used in the study.

The first type was ActiGraph (AG). This type of accelerometer was formally known as Computer Science and Applications (CSA) from Manufacturing Technology Inc. (ActiGragh, LLC, Fort Walton Beach, FL, USA). It is designed to be worn on the right side of the body on the hip. The second type was ActivPAL™ series 6.2 (AP) from PAL technologies Ltd, Glasgow, Scotland, UK. It is 5.4cm (L) x 3.5cm (W). It is worn on the middle anterior aspect of the right thigh. The third type was SenseWear PRO_2 (SW) Armband manufactured by BodyMedia Inc™, USA. It is designed to be worn on the back of the right upper arm (the triceps muscle). Body movements were measured simultaneously using all three types of accelerometers, (ActivPAL, ActiGraph and Sensewear Pro_2) worn on the thigh, hip and upper arm, respectively. Stride frequency of the participants was monitored during exercise (averaged over the last 2 minutes of each work load). The third session took place one or two weeks after the second session. Participants during this visit repeated the same procedure that was performed during the second visit.

2.2.3 Data collection and analysis:

All data from the three accelerometers, oxygen consumption and heart rate monitors was entered in MS Excel files as well as body measurements. To enable comparison of data from all devices the data was examined as energy expenditure (kcal.min^{-1}).

Data obtained from the ActiGraph included counts per 15sec., kcal.min^{-1} and stride frequency. EE was calculated using the published Freedson equation (Freedson *et al.*, 1998):

$$\text{Kcal.min}^{-1} = (0.00094 \times \text{counts.min}^{-1}) + (0.1346 \times \text{body mass (Kg)}) - 7.37418$$

Data obtained from ActivPAL included energy expenditure every 15sec. The energy expenditure was calculated by the device using the following equation:

$$\text{EE (MET.h)} = (1.4 \times \text{activity duration(hrs)}) + (4 - 1.4) \times (\text{steps/min})/120 \times \text{activity duration}$$

Energy expenditure (kcal.min^{-1}) was then calculated by summing every four readings of (MET/15sec.) then multiplying the result (MET/min) by 3.5 (1MET=3.5Kcal for adults) and by 4.15Kcal for adolescents (Harrell *et al.*, 2005).

All SenseWear data including energy expenditure (kcal.min^{-1}) was obtained from the device using software PRO$_2$ version 6.1.

Energy expenditure (kcal.min^{-1}) and VO_2 data from indirect calorimetry was measured during the experimental tests using online gas analysis. Heart rate data was measured by HR monitor model Polar S-625X^{TM}. Heart rate data was averaged over the last 2 minutes for each work load.

2.2.4 Statistical analysis:

Data are summarised as means ±SD. Reliability and validity were analysed using SPSS software version 15.0 for Windows. Reliability was examined using paired-samples T-tests comparing 1st test to the 2nd test one or two weeks apart. Intraclass coefficient correlation (ICC) was also used to assess the correlation between scores value in 1st vs. 2nd tests. Coefficients of variation (CV) was used to obtain the variation of the scores value between 1st and 2nd tests.

Validity was examined using paired-samples T-tests comparing 2nd test data for each device with the 2nd test values obtained from indirect calorimetry. Additionally, intraclass coefficient correlation (ICC) was used to assess the correlation between each device output (in kcals.min^{-1}) versus the values obtained from indirect calorimetry in the 2nd test.

2.3 Results

Due to the volume of data obtained in the present study the results are reported separately for reliability and validity and also separately for adults and adolescents.

2.3.1 Reliability - Adults

All accelerometers were reliable on the flat across all speeds for adults. There was no significant difference between caloric cost of activity in the first and

second tests on the flat across all speeds for any of the three accelerometers. Moreover, data from ActiGraph (AG), ActivPAL (AP) and SenseWear (SW) showed strong intra-instrument reliability on the flat, (ICC) r= 0.99, r= 0.99 and r= 0.94 all at p<0.001, respectively. Coefficients of variation (CV) showed only small variations between trials in AG and AP but relatively larger between trial variation for SW. These results are summarised in Table 2-3.

Table 2-3 T-test, Reliability, on the flat for all groups across all speeds, n= 61 adults

All groups (Lean and obese/overweight) (kcals.min^{-1})	**Paired Differences**	**95% Confidence interval of difference**		**Sig. (2-tailed)**	**CV(%)**
	Mean ±SD	**Lower**	**Upper**		
EE AG1- EE AG2	0.018 ±0.18	-0.008	0.044	0.19	3.2%
EE AP1- EE AP2	-0.0007 ±0.17	-0.025	0.024	0.96	3.5%
EE SW1-EE SW2	0.060 ±0.86	-0.185	0.066	0.35	15.0%

All accelerometer devices were reliable on an incline (5% gradient) across all speeds. Table 2-4 shows more details on reliability between trials at a 5% gradient across all speeds for all groups.

Table 2-4 T-test, Reliability, incline (5% gradient) for all groups across all speeds n= 61 adults

All groups (Lean and obese/overweight) (kcals.min^{-1})	**Paired Differences**	**95% Confidence interval of difference**		**sig. (2-tailed)**	**CV(%)**
	Mean ±SD	**Lower**	**Upper**		
EE AG1- EE AG2	-0.013 ±0.13	-0.006	0.0319	0.18	2.1%
EE AP1- EE AP2	0.009 ±0.15	-0.013	0.031	0.42	3.2%
EE SW1-EE SW2	0.063 ±0.72	-0.041	0.168	0.24	12.2%

Also, data showed high correlation between trials at the 5% gradient across all speeds. ActiGraph, ActivPAL and SenseWear gave strong intra-instrument reliability (ICC) r= 0.99, r= 0.99 and r= 0.96 all at p<0.001, respectively. The reliability of these accelerometers is clearly illustrated in Figure 2.3.

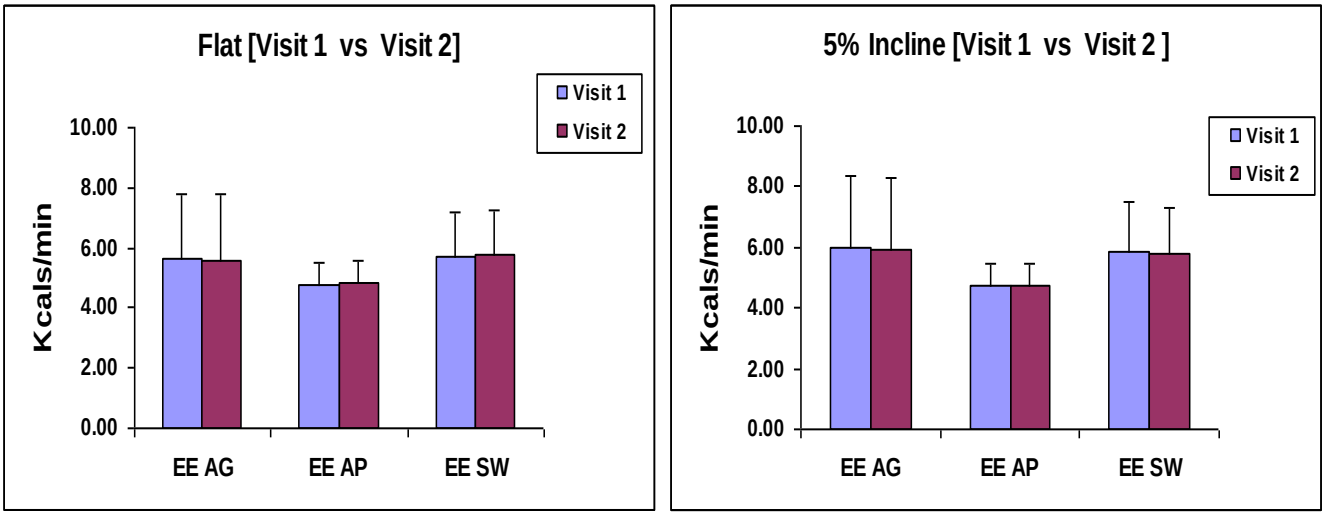


Figure 2.3 Reliability for lean and overweight adults observed in both gradients, n= 61

2.3.2 Reliability - Adolescents

All accelerometers were found to be reliable for both gradients across all speeds for adolescents. Table 2-5 shows that there was no significant difference between caloric expenditure estimates for the first and second tests on the flat across all speeds for any of the three accelerometers. The coefficients of variation (CV) between trials was higher in AG and lower in AP whereas SW reported intermediate CV between trials. On the other hand, data from ActiGraph, ActivPAL and SenseWear showed strong intra-instrument reliability on the flat, (ICC) r= 0.98, r= 0.98 and r= 0.95 all at $p<0.001$ respectively.

Table 2-5 T-test, Reliability, on the flat across all speeds, n= 29

Adolescents (kcals.min^{-1})	**Paired Differences**	**95% Confidence interval of difference**		**Sig. (2-tailed)**	**CV(%)**
	Mean ±SD	**Lower**	**Upper**		
EE AG1- EE AG2	-0.085 ±0.47	-0.186	0.016	0.10	16.9%
EE AP1- EE AP2	-0.0002 ±0.17	-0.036	0.036	0.99	4.9%
EE SW1-EE SW2	0.078 ±0.57	-0.043	0.2	0.20	11.4%

Furthermore, all accelerometers were reliable on an incline (5% gradient) across all speeds. Table 2-6 shows more details on reliability between trials at 5% gradient across all speeds.

Table 2-6 T-test, Reliability, incline (5% gradient) across all speeds n= 29

Adolescents (kcals.min^{-1})	Paired Differences	95% Confidence interval of difference		sig. (2-tailed)	CV(%)
	Mean ±SD	Lower	Upper		
EE AG1- EE AG2	-0.061 ±0.32	-0.009	0.13	0.09	11.4%
EE AP1- EE AP2	0.003 ±0.16	-0.031	0.037	0.86	4.6%
EE SW1-EE SW2	0.019 ±0.52	-0.091	0.13	0.73	9.9%

Moreover, reliability assessment using intraclass correlation coefficient analysis proved that all accelerometers devices, ActiGraph, ActivPAL and SenseWear had strong intra-instrument reliability between the two tests at 5% uphill across all speeds, ICC r =0.99, r= 0.96 and r= 0.96 all at p<0.001 respectively. Figure 2.4 clearly shows that all these devices were reliable in adolescents in both gradients.

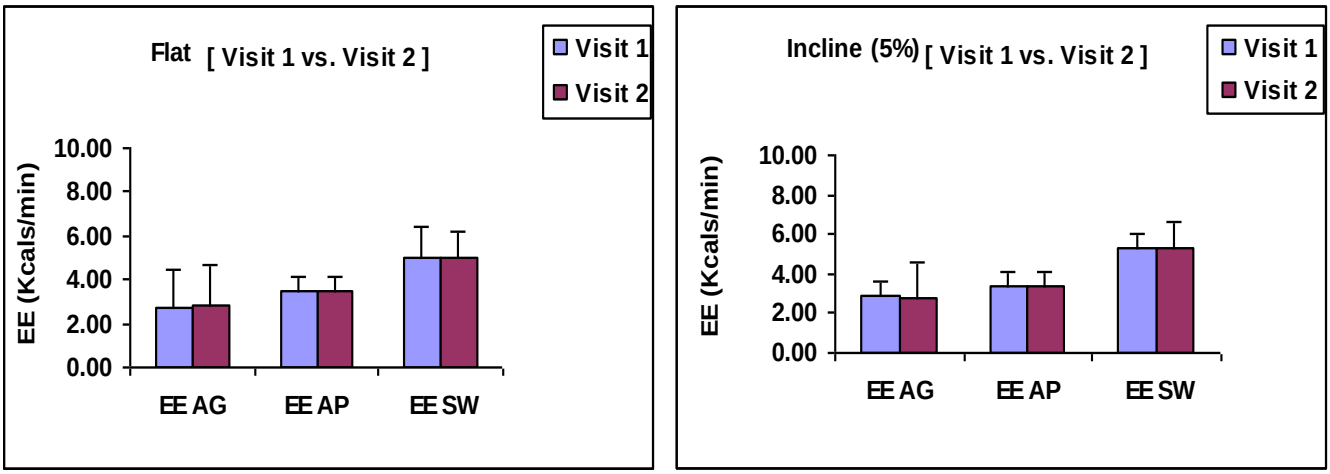


Figure 2.4 Reliability for adolescents group observed in both gradients

2.3.3 Validity - Adults

In contrast to the reliability, data indicate that all devices had problems with validity during flat and incline walking (Table 2.7).

Table 2.7 Comparison of mean (SD) energy expenditure from accelerometers with data obtained using indirect calorimetry adults. (lean n=30, overweight/obese n=31).

Gradient - Group	**Speeds**	**IC** Kcals.min^{-1}	**AG** Kcals.min^{-1}	**AP** Kcals.min^{-1}	**SW** Kcals.min^{-1}
Flat – Lean	3 Km.h^{-1}	3.17+0.53	2.41+1.22*	3.73+0.42*	4.17+0.8*
	4.5 Km.h^{-1}	4.05+0.64	4.11+1.28	4.13+0.45	4.76+0.87*
	6 Km.h^{-1}	5.59+0.88	5.79+1.39	4.46+0.47*	5.84+0.97
Flat – Overweight	3 Km.h^{-1}	3.84+0.67	5.53+2.12*	5.01+0.81*	5.35+1.42*
	4.5 Km.h^{-1}	5.08+0.90	7.09+2.13*	5.50+0.85*	6.45+1.40*
	6 Km.h^{-1}	7.29+1.19	8.55+2.20*	5.92+0.91*	7.94+1.84*
Incline – Lean	3 Km.h^{-1}	3.99+0.70	2.85+1.24*	3.63+0.41*	4.03+0.73
	4.5 Km.h^{-1}	5.53+0.90	4.43+1.29*	4.07+0.48*	4.73+0.89*
	6 Km.h^{-1}	7.77+1.19	6.00+1.46*	4.45+0.48*	5.96+1.00*
Incline–Overweight	3 Km.h^{-1}	5.17+0.96	5.94+2.13*	4.88+0.78*	5.45+1.23
	4.5 Km.h^{-1}	7.18+1.25	7.51+2.10	5.41+0.84*	6.67+1.53*
	6 Km.h^{-1}	9.92+1.64	8.93+2.21*	5.91+0.91*	8.05+1.59*

***** Indicate significant differences from IC *P*<0.05

IC: indirect calorimetry, AG: ActiGraph, AP: ActivPAL, SW: SenseWear

Data analysis revealed that AG was the best to assess EE during normal walking (4.5Km.h^{-1}) and brisk walking (6Km.h^{-1}) for lean adults on the flat at p=0.75 and p=0.29 respectively. On the other hand, SW was the most valid for slow walking at 3 Km.h^{-1} on an incline for both lean and overweight/obese groups (lean, P=0.68 and overweight, P=0.10). AP was more accurate for lean

on the flat at moderate walking (4.5Km.h^{-1}) p=0.40. However, AG is likely to underestimate the EE in lean at slow walking (3Km.h^{-1}) on the flat and during all speeds on an incline. In addition, AG also underestimated EE in overweight/obese group at brisk walking (6Km.h^{-1}) on an incline. In contrast, AG overestimated EE in overweight/obese group during all walking speeds on the flat as well as during slow walking on an incline. These findings are illustrated in Figure 2.4.

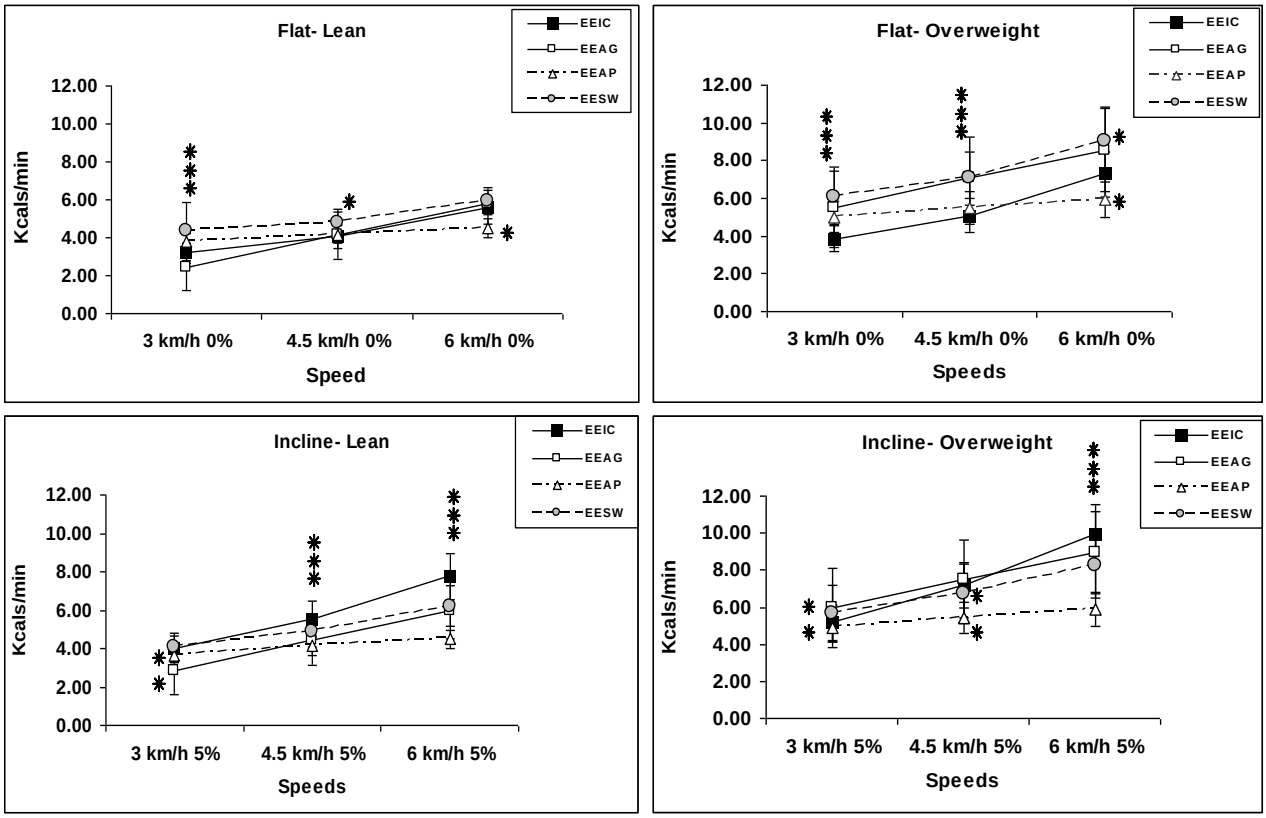
Data from the devices was also compared to the gold standard measurement (indirect calorimetry) using Intraclass Coefficient Correlation (ICC). Table 2.8 shows that AG, AP and SW have good to high correlation with indirect calorimetry across all speeds and during each speed separately for both groups in both gradients (r= between 0.55 – 0.92).

Table 2.8 Interclass Coefficient Correlation, r (CI) of ActiGraph (AG), ActivPAL (AP) and SenseWear (SW) comparison to Indirect Calorimetry (IC) in adults. (lean n=30, overweight/obese n=31).

Gradient - Group	**Speeds**	**AG r (CI)**	**AP r (CI)**	**SW r (CI)**
Flat – Lean	All speeds	0.88 (0.82 – 0.92)**	0.71 (0.56 - 081)**	0.85 (0.78 - 0.90)**
	3 $Km.h^{-1}$	0.69 (-0.36 – 0.85)**	0.71 (0.4 – 0.86)**	0.61 (0.17 - 0.81)*
	4.5 $Km.h^{-1}$	0.72 (0.41 – 0.87)**	0.77 (0.51 – 0.90)**	0.67 (0.30 – 0.84)*
	6 $Km.h^{-1}$	0.74 (0.46 – 0.88)**	0.72 (0.42 – 0.87)**	0.72 (0.42 – 0.87)**
Flat – Overweight	All speeds	0.82 (0.73 – 0.88)**	0.75 (0.62 - 083)**	0.83 (0.75 – 0.89)**
	3 $Km.h^{-1}$	0.60 (0.17 – 0.81)*	0.89 (0.76 – 0.95)**	0.55 (0.06 – 0.78)*
	4.5 $Km.h^{-1}$	0.67 (0.32 – 0.84)**	0.88 (0.75 - 0.94)**	0.61 (0.18 – 0.81)*
	6 $Km.h^{-1}$	0.72 (0.43 – 0.87)**	0.85 (0.69 - 0.93)**	0.72 (0.41 – 0.86)**
Incline – Lean	All speeds	0.92 (0.88 – 0.95)**	0.61 (0.41 – 0.75)**	0.85 (0.78 – 0.90)**
	3 $Km.h^{-1}$	0.80 (-0.58 – 0.91)**	0.77 (0.51 – 0.89)**	0.84 (0.67 – 0.92)**
	4.5 $Km.h^{-1}$	0.84 (0.66 – 0.92)**	0.73 (0.44 – 0.87)**	0.81 (0.60 – 0.91)**
	6 $Km.h^{-1}$	0.78 (0.53 – 0.89)**	0.61 (0.18 – 0.81)*	0.70 (0.36 – 0.86)**
Incline – Overweight	All speeds	0.87 (0.80 – 0.91)**	0.69 (0.53 – 0.79)**	0.85 (0.77 – 0.90)**
	3 $Km.h^{-1}$	0.76 (0.50 – 0.88)**	0.91 (0.81 - 0.96)**	0.81 (0.61 – 0.91)**
	4.5 $Km.h^{-1}$	0.81 (0.60 – 0.91)**	0.86 (0.70 - 0.93)**	0.74 (0.47 – 0.88)**
	6 $Km.h^{-1}$	0.79 (0.57 – 0.90)**	0.79 (0.56 – 0.90)**	0.73 (0.45 – 0.87)**

* Indicate that there is significant correlation at $P<0.05$

** Indicate that there is significant correlation at $P\leq0.001$



***** indicate significant differences from (IC), *P*<0.05.

Figure 2.4 Validity of accelerometers comparing to indirect calorimetry (IC), in adults, (lean n=30, overweight/obese n=31)..

Multiple comparison one-way ANOVA was used to examine the ability of these devices (AG, AP and SW) to detect EE differences between speeds. The results showed that AG and SW were able to detect EE differences between all speeds for both groups in both gradients. On the other hand, AP could detect the differences between all speeds in lean group in both gradients but it was not sensitive enough to detect EE differences in overweight/obese group neither between slow and normal walking speed nor between normal and brisk walking speed in both gradients. Statistically, mean (SD) differences showed that AG was significantly more sensitive to detect EE differences on flat for lean

group between slow walking (3km.h^{-1})=2.41(1.22) and normal walking (4.5km.h^{-1})= 4.11(1.29), (-1.69) p<0.001, and between normal walking= 4.11(1.29) and brisk walking (6km.h^{-1})= 5.79(1.39), (-1.69) p<0.001. Also, the sensitivity to detect differences between speeds was also observed in overweight/obese adults between slow= 5.53(2.12) and normal= 7.09(2.13), (-1.57) p=0.019 and between normal= 7.09(2.13) and brisk= 8.55(2.20), (-1.45) p=0.033. The same result was shown by AG for walking on an incline for lean between slow= 2.85(1.24) and normal= 4.43(1.29), (-1.59) p<0.001 and between normal= 4.43(1.29) and brisk= 6.00(1.46), (-1.56) p<0.001. Data from overweight/obese shows that AG was also sensitive enough to detect EE between slow= 5.94(2.13) and normal= 7.51(2.10), (-1.57) p=0.019 and between normal= 7.51(2.10) and brisk= 8.93(2.21), (-1.42) p=0.037. Likewise, SW seems to be good device in terms of detecting EE differences between speeds in both groups in both gradients (*P* value between 0.001-0.04). However, AP was only sensitive to detect EE between walking speeds in lean in both gradients (*P* value between 0.002-0.04).

2.3.4 Validity – Adolescents

Comparison of means (SD) energy expenditure between accelerometers and indirect calorimetry is summarised in Table 2.9. Results indicate that all devices had problems with validity on flat during walking except ActivPAL (AP) at 3 Km.h^{-1} mean SD = 3.12$\pm$0.52 compared to the indirect calorimetry with *P*= 0.20.

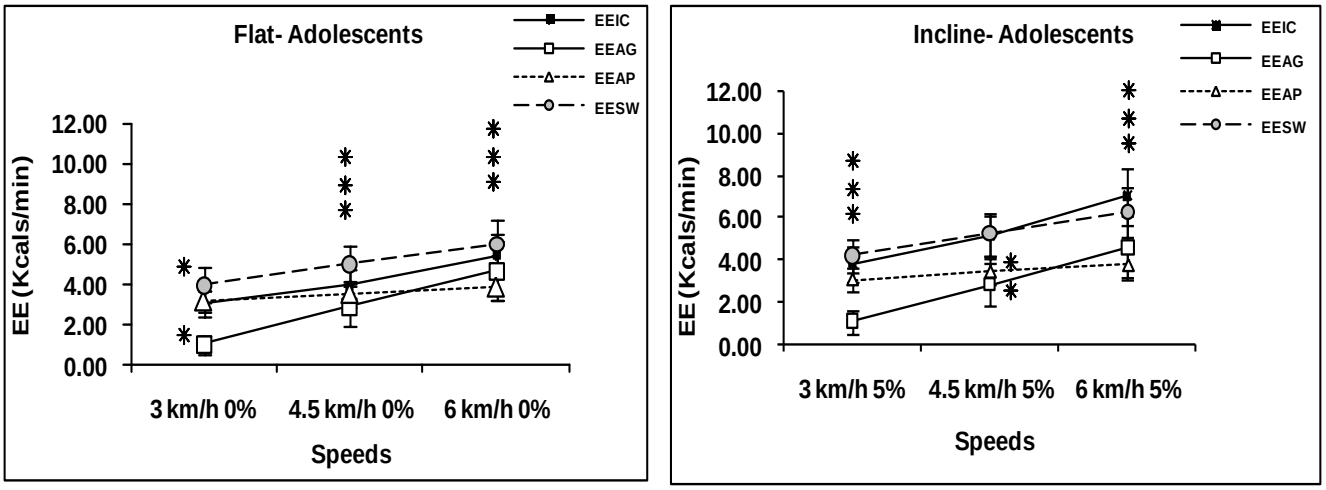
Table 2.9 Comparing means (SD) of EE (Kcals.min^{-1}) assessed by accelerometers with indirect calorimetry in adolescents n=29 (male=15, female=14)..

Gradient	**Speeds**	**IC** Kcals.min^{-1}	**AG** Kcals.min^{-1}	**AP** Kcals.min^{-1}	**SW** Kcals.min^{-1}
All Adolescents – Flat	3 Km.h^{-1}	3.00±0.69	0.98±0.50*	3.12±0.52	3.91±0.87*
	4.5 Km.h^{-1}	3.90±0.81	2.87±1.00*	3.49±0.60*	4.96±0.92*
	6 Km.h^{-1}	5.40±1.06	4.63±1.45*	3.80±0.66*	5.97±1.16*
Male – Flat	All speeds	4.17±1.30	2.93±1.88*	3.40±0.72*	4.76±1.39*
Female – Flat	All speeds	4.04±1.34	2.72±1.78*	3.54±0.57*	5.15±1.16*
All Adolescents – Incline	3 Km.h^{-1}	3.83±0.83	1.08±0.55*	3.05±0.53*	4.22±0.78*
	4.5 Km.h^{-1}	5.15±1.00	2.82±0.98*	3.44±0.59*	5.24±1.02
	6 Km.h^{-1}	7.03±1.37	4.58±1.49*	3.77±0.65*	6.28±1.22*
Male – Incline	All speeds	5.48±1.80	2.83±1.82*	3.35±0.72*	5.19±1.41
Female – Incline	All speeds	5.18±1.61	2.82±1.77*	3.49±0.58*	5.31±1.22

***** indicate significant differences from IC *P* <0.05

IC: indirect calorimetry, AG: ActiGraph, AP: ActivPAL, SW: SenseWear

Figure 2.5 shows that ActiGraph (AG) and ActivPAL both significantly underestimated EE on flat across all speeds where SenseWear (SW) overestimated EE. On an incline, SenseWear was the only one that could accurately detect EE during normal walking speed (4.5Km.h^{-1}) mean SD= 5.24±1.02 with p= 0.56 and at overall speeds for both male and female adolescents groups, mean SD= 5.19±1.41 with p= 0.08 and mean SD= 5.31±1.22 with p= 0.30 respectively.



* indicate significant differences from indirect calorimetry, p<0.05.

Figure 2.5 Validity of accelerometers comparing to indirect calorimetry (IC), n= 29 (male=15, female=14).

Data from the accelerometers was also compared with that obtained from indirect calorimetry gas analysis using Intraclass Coefficient Correlation (ICC) and 95% confidence interval (95%CI). Table 2.10 shows that all devices (AG AP and SW) have high to excellent correlation with indirect calorimetry (IC) across all speeds in both gradients for all adolescents as a group and for each gender (males and females) separately, (r= between 0.68 – 0.95, p<0.05).

Table 2.10 Intraclass Coefficient Correlation, r(CI) of ActiGraph (AG), ActivPAL (AP) and SenseWear (SW) comparison to Indirect Calorimetry(IC), adolescents n=29 (Male=15, Female=14)

Gradient - Group	**Speeds**	**AG r (CI)**	**AP r (CI)**	**SW r (CI)**
All Children – Flat	All speeds	0.87 (0.80 – 0.91)**	0.75 (0.62 – 084)**	0.85 (0.77 – 0.90)**
	3 Km.h^{-1}	0.30 (-0.48 – 0.67)	0.82 (0.62 – 0.92)**	0.09 (-0.94 – 0.57)
	4.5 Km.h^{-1}	0.62 (0.19 – 0.82)*	0.83 (0.63 – 0.92)**	0.70 (0.36 – 0.86)**
	6 Km.h^{-1}	0.78 (0.52 – 0.90)**	0.81 (0.57 – 0.91)**	0.83 (0.64 – 0.92)**
Male – Flat	All speeds	0.84 (0.71 – 0.91)**	0.79 (0.62 – 089)**	0.83 (0.69 – 0.91)**
Female – Flat	All speeds	0.90 (0.81 – 0.94)**	0.71 (0.47 – 0.85)**	0.90 (0.81 – 0.95)**
All Children – Incline	All speeds	0.92 (0.88 – 0.95)**	0.68 (0.51 – 0.79)**	0.88 (0.82 – 0.92)**
	3 Km.h^{-1}	0. 23 (-0.64 – 0.64)	0.81 (0.60 – 0.91)**	0.52 (-0.02 – 0.78)*
	4.5 Km.h^{-1}	0.80 (0.57 – 0.83)**	0.83 (0.64 - 0.92)**	0.84 (0.65 – 0.92)**
	6 Km.h^{-1}	0.85 (0.68 – 0.93)**	0.73 (0.42 - 0.87)**	0.86 (0.69 – 0.93)**
Male – Flat	All speeds	0.90 (0.82 – 0.95)**	0.70 (0.46 – 0.84)**	0.87 (0.76 – 0.93)**
Female – Flat	All speeds	0.95 (0.91 – 0.97)**	0.68 (0.40 – 0.83)**	0.92 (0.85 – 0.96)**

* Indicate that there is significant correlation at $P<0.05$

** Indicate that there is significant correlation at $P<0.001$

The multiple comparison one-way ANOVA was also used to examine the ability of these devices (AG, AP and SW) to detect EE differences between different

walking speeds. The results in the current study indicated that AG was sensitive to detect EE differences between all speeds for all adolescents as a group and for males and females separately in both gradients. On the other hand, SW could detect EE between speeds in adolescents as a group in both gradients as well as in female group on an incline, but could not detect EE differences between speeds on some occasions in male group in both gradients and in female group on the flat. In contrast, AP could not detect the EE differences between all speeds neither in adolescents as a group nor in each gender in both gradients except between slow walking and brisk walking speeds (3 $Km.h^{-1}$ – 6 $Km.h^{-1}$).

Statistically, mean (SD), (mean differences), showed that AG was significantly sensitive to detect EE differences on flat for adolescents as a group between slow walking (3$km.h^{-1}$)= 0.98(0.50) and normal walking (4.5$km.h^{-1}$)= 2.87(1.00), (-1.89) $p<0.001$, and between normal walking= 2.87(1.00) and brisk walking (6$km.h^{-1}$)= 4.63(1.45), (-1.75) $p<0.001$. On an incline, AG was able to detect EE differences between speeds in adolescents as a group between slow= 1.08(0.55) and normal= 2.82(0.98), (-1.73) $p<0.001$ and between normal= 2.82(0.98) and brisk= 4.58(1.49), (-1.76) $p<0.001$. Moreover, AG was also able to detect EE differences between speeds in each gender group on the flat and at 5% incline ($P \leq 0.003$).

SW seems sensitive enough to detect EE differences between walking speeds in adolescents as a group in both gradients. The results of SW device indicated significant differences between slow walking= 3.91(0.87) and normal= 4.96(0.92), (-1.05) p=0.001 and between normal= 4.96(0.92) and brisk=

4.95(1.30), (-1.01) p=0.001 on the flat. On an incline (5% grade), EE differences between walking speeds were detected by SW between slow= 4.22(0.78) and normal= 5.24(1.02), (-1.02) p=0.001 and between normal= 5.24(1.02) and brisk= 5.25(1.32), (-1.05) p=0.001. However, when SW data of each gender (male group and female group) was used, SW failed to detect EE differences between some of the walking speeds in some occasions. In male group, SW was only able to detect EE differences between slow and normal walking speeds in both gradients (P= between 0.01–0.04). In female group, SW was able to detect EE differences between walking speeds in both gradients (P= between 0.01–0.03) except between slow and normal walking speeds on the flat (P=0.12).

In contrast, AP could not detect EE differences between walking speeds neither in adolescents as a group nor in each gender group in both gradients. Data obtained from AP showed that the EE differences between walking speeds were not significant in adolescents as a group in both gradients (P= between 0.05–0.15). Similar results were found in male group in both gradients (P= between 0.36–0.53) and in female group in both gradients (P= between 0.11–0.22).

Summary of the reliability and validity results for both adults and adolescents are illustrated in Table 2.11.

Table 2.11 Summary of the reliability and validity for both adults n=61 and adolescents n=29.

Reliability							
Groups	**Devices**	**Flat**			**Incline**		
		Across all speeds			**Across all speeds**		
Adults	**AG**	✓			✓		
	AP	✓			✓		
	SW	✓			✓		
Adolescents	**AG**	✓			✓		
	AP	✓			✓		
	SW	✓			✓		
Validity							
Groups	**Devices**	**Flat**			**Incline**		
		3Km.h^{-1}	4.5Km.h^{-1}	6Km.h^{-1}	3Km.h^{-1}	4.5Km.h^{-1}	6Km.h^{-1}
Adults (Lean)	**AG**	✖	✓	✓	✖	✖	✖
	AP	✖	✓	✖	✖	✖	✖
	SW	✖	✖	✓	✓	✖	✖
Adults (Overweight/obese)	**AG**	✖	✖	✖	✖	✓	✖
	AP	✖	✖	✖	✖	✖	✖
	SW	✖	✖	✖	✓	✖	✖
Adolescents	**AG**	✖	✖	✖	✖	✖	✖
	AP	✓	✖	✖	✖	✖	✖
	SW	✖	✖	✖	✖	✓	✖

(✓) means that the device was reliable/valid.

(✖) means that the device was **not** reliable/valid.

2.3.5 Heart rate measurement and ActiGraph – Adults

One-way ANOVA (multiple comparisons) was used to determine change in heart rate over different walking speeds. All participants in both groups showed significant differences in heart rate (HR) between speeds in both gradients. Table 2.12 summarise this finding.

Table 2.12 Comparing Mean(SD) and mean differences of HR between speeds in adults, n= 61 (lean=30, overweight/obese=31).

Level- Group	Speeds	Mean(SD)	Mean Differences	*P* value
Flat – Lean	3 - 4.5 Km.h^{-1}	84.07(8.70) - 92.43(9.96)	8.367*	0.007
	4.5 - 6 Km.h^{-1}	92.43(9.96) - 105.10(11.16)	12.667**	0.000
	3 - 6 Km.h^{-1}	84.07(8.70) - 105.10(11.16)	21.033**	0.000
Flat – Overweight	3 - 4.5 Km.h^{-1}	88.81(11.70) - 98.03(12.23)	9.226*	0.027
	4.5 - 6 Km.h^{-1}	98.03(12.23) - 115.06(15.45)	17.032**	0.000
	3 - 6 Km.h^{-1}	88.81(11.70) - 115.06(15.45)	26.258**	0.000
Incline – Lean	3 - 4.5 Km.h^{-1}	89.93(9.34) - 103.33 (10.95)	13.400**	0.000
	4.5 - 6 Km.h^{-1}	103.33 (10.95) - 121.70(14.36)	18.367**	0.000
	3 - 6 Km.h^{-1}	89.93(9.34) - 121.70(14.36)	31.767**	0.000
Incline – Overweight	3 - 4.5 Km.h^{-1}	98.74(14.10) - 113.13(15.08)	14.387**	0.001
	4.5 - 6 Km.h^{-1}	113.13(15.08) - 133.81(15.89)	20.677**	0.000
	3 - 6 Km.h^{-1}	98.74(14.10) - 133.81(15.89)	35.065**	0.000

* Indicate significant differences at $P<0.05$

** Indicate significant differences at $P\leq0.001$

In this study, EE was assessed during the laboratory exercise test using different types of accelerometer devices including ActiGraph as well as heart rate monitor. Physical activity energy expenditure, as a result of physiological and locomotor components, can be accurately assessed by combining heart rate (HR) monitor data with accelerometer device (ActiGraph). In the laboratory exercise test, the counts per minute (CPM) of ActiGraph were combined with the heart rate to assess EE precisely. The combination was done by dividing CPM by HR values ($CPM.HR^{-1}$) for each workload for both groups in both gradients. The mean (SD) and mean differences of the obtained value of CPM per heart beat ($CPM.b^{-1}$) is summarised in Table 2.13.

Table 2.13 Comparing mean(SD) and mean differences of the ActiGraph's counts per minutes per beat (CPM.b^{-1}) in adults (lean=30, overweight/obese=31).

Level- Group	Speeds	Mean(SD)	Mean Differences
Flat – Lean	3 - 4.5 Km.h^{-1}	11.83(3.98) - 30.65(6.78)	-18.82*
	4.5 - 6 Km.h^{-1}	30.65(6.78) - 44.11(9.33)	-13.46*
	3 - 6 Km.h^{-1}	11.83(3.98) - 44.11(9.33)	-32.28*
Flat – Overweight	3 - 4.5 Km.h^{-1}	13.78(4.66) - 28.45(7.35)	-14.67*
	4.5 - 6 Km.h^{-1}	28.45(7.35) - 38.15(10.82)	-9.7*
	3 - 6 Km.h^{-1}	13.78(4.66) - 38.15(10.82)	-24.37*
Incline – Lean	3 - 4.5 Km.h^{-1}	16.5(5.2) - 30.87(6.71)	-14.37*
	4.5 - 6 Km.h^{-1}	30.87(6.71) - 40.42(8.5)	-9.55*
	3 - 6 Km.h^{-1}	16.5(5.2) - 40.42(8.5)	23.92*
Incline – Overweight	3 - 4.5 Km.h^{-1}	16.47(4.32) - 28.25(6.5)	-11.78*
	4.5 - 6 Km.h^{-1}	28.25(6.5) - 34.54(9.56)	-6.28*
	3 - 6 Km.h^{-1}	16.47(4.32) - 34.54(9.56)	-18.06*

* Indicate significant differences at $P < .005$

Although CPM.b^{-1} detected the differences between speeds for both groups in both gradients, the correlation between CPM.b^{-1} and indirect calorimetry (IC) using intraclass correlation coefficient ICC was somewhat high on flat for lean and overweight/obese groups as r(CI)=0.81 (0.7–0.87), P=0.996 and r(CI)=0.75 (0.62–0.83), P= 0.996 respectively. Yet, ICC was low on an incline for both groups lean and overweight/obese r(CI)=0.2 (-0.22–0.47), P<.001, r(CI)=0.27 (-0.1–0.52), P<0.001 respectively.

2.3.6 Heart rate measurement and ActiGraph – Adolescents

One-way ANOVA (multiple comparisons) also used to determine change in heart rate (HR) over different walking speeds in adolescents group. All participants in adolescents as a group showed significant differences in heart rate (HR) between speeds in both gradients. Heart rate measurement in male and female groups separately also showed significant differences between all speeds on uphill gradient. However, on the flat, HR in both genders indicated that there were no significant differences between slow waling (3Km.h^{-1}) and normal walking (4.5 Km.h^{-1}) speeds for both gender. Table 2.14 summarises these results.

Table 2.14 Comparing Mean(SD) and mean differences of HR between speeds in adolescents n=29 (male=15, female=14)

Level- Group	**Speeds**	**Mean(SD)**	**Mean Differences**	***P Value***
Flat – All adolescents	3 - 4.5 Km.h^{-1}	92.7(12.1) – 103.6(13.0)	10.93*	0.017
	4.5 - 6 Km.h^{-1}	103.6(13.0) – 119.4(17.1)	15.76**	0.000
	3 - 6 Km.h^{-1}	92.7(12.1) – 119.4(17.1)	26.69**	0.000
Flat – Male	3 - 4.5 Km.h^{-1}	88.9(12.5) – 98.6(13.4)	9.73	0.153
	4.5 - 6 Km.h^{-1}	98.6(13.4) – 112.0(14.4)	13.40*	0.033
	3 - 6 Km.h^{-1}	88.9(12.5) – 112.0(14.4)	23.13*	0.000
Flat – Female	3 - 4.5 Km.h^{-1}	96.8(10.6) – 109.0(10.5)	12.21	0.055
	4.5 - 6 Km.h^{-1}	109.0(10.5) – 127.3(16.6)	18.29*	0.002
	3 - 6 Km.h^{-1}	96.8(10.6) – 127.3(16.6)	30.50**	0.000
Incline- All adolescents	3 - 4.5 Km.h^{-1}	101.8(13.2) – 116.5(15.3)	14.69*	0.004
	4.5 - 6 Km.h^{-1}	116.5(15.3) – 136.0(20.0)	19.52**	0.000
	3 - 6 Km.h^{-1}	101.8(13.2) – 136.0(20.0)	34.21**	0.000
Incline – Male	3 - 4.5 Km.h^{-1}	96.1(11.9) – 109.1(12.8)	12.93*	0.035
	4.5 - 6 Km.h^{-1}	109.1(12.8) – 124.7(14.5)	15.60*	0.009
	3 - 6 Km.h^{-1}	96.1(11.9) – 124.7(14.5)	28.53**	0.000
Incline – Female	3 - 4.5 Km.h^{-1}	107.9(11.9) – 124.4(14.00)	16.65*	0.020
	4.5 - 6 Km.h^{-1}	124.4(14.00) – 148.1(20.0)	23.71**	0.001
	3 - 6 Km.h^{-1}	107.9(11.9) – 148.1(20.0)	40.29**	0.000

* Indicate significant differences at $P<0.05$

** Indicate significant differences at $P\leq0.001$

In adolescents the counts per minute (CPM) of ActiGraph were also combined with the heart rate to assess whether EE obtained from the combination of AG and HR can provide better EE prediction comparing to the estimated EE obtained from AG alone. The combination was done ($CPM.HR^{-1}$) for each workload for adolescents as a group and for each gender separately in both gradients. The mean (SD) and mean differences of the score values of $CPM.b^{-1}$ is summarised in Table 2.15. $CPM.b^{-1}$ detected the differences between speeds for all adolescents as a group and for each gender separately in both gradients except in female group on an incline between normal and brisk walking speeds. Yet, the correlation between $CPM.b^{-1}$ and indirect calorimetry (IC) using intraclass correlation coefficient ICC was low in adolescents as a group and in each gender separately in both gradients. The recorded ICC was between r(CI)= 0.159(-0.53 – 0.54) and r(CI)= 0.357(-0.20 – 0.66) and all values were at $P< 0.001$.

Table 2.15 Comparing mean(SD) and mean differences of the ActiGraph's counts per minutes per beat (CPM.b^{-1}) between speeds in adolescents n=29 (male=15, female=14)

Level- Group	**Speeds**	**Mean(SD)**	**Mean Differences**
Flat – All adolescents	3 - 4.5 Km.h^{-1}	12.83(2.47) – 26.86(1.67)	-14.03*
	4.5 - 6 Km.h^{-1}	26.86(1.67) – 37.45(2.03)	10.59**
	3 - 6 Km.h^{-1}	12.83(2.47) – 37.45(2.03)	24.62*
Flat – Male	3 - 4.5 Km.h^{-1}	15.67(17.54) – 29.62(8.00)	13.95**
	4.5 - 6 Km.h^{-1}	29.62(8.00) – 42.44(9.11)	12.81**
	3 - 6 Km.h^{-1}	15.67(17.54) – 42.44(9.11)	26.77*
Flat – Female	3 - 4.5 Km.h^{-1}	9.79(5.45) – 23.90(9.35)	14.11*
	4.5 - 6 Km.h^{-1}	23.90(9.35) – 32.11(10.46)	8.20**
	3 - 6 Km.h^{-1}	9.79(5.45) – 32.11(10.46)	22.31*
Incline – All adolescents	3 - 4.5 Km.h^{-1}	11.47(5.82) – 24.90(8.41)	13.43*
	4.5 - 6 Km.h^{-1}	24.90(8.41) – 33.60(9.95)	8.70*
	3 - 6 Km.h^{-1}	11.47(5.82) – 33.60(9.95)	22.13*
Incline – Male	3 - 4.5 Km.h^{-1}	12.00(6.28) – 27.25(7.92)	15.26*
	4.5 - 6 Km.h^{-1}	27.25(7.92) – 37.63(8.17)	10.38**
	3 - 6 Km.h^{-1}	12.00(6.28) – 37.63(8.17)	25.64*
Incline – Female	3 - 4.5 Km.h^{-1}	10.90(5.46) – 22.37(8.46)	11.48**
	4.5 - 6 Km.h^{-1}	22.37(8.46) – 29.28(10.12)	6.91
	3 - 6 Km.h^{-1}	10.90(5.46) – 29.28(10.12)	18.38*

* Indicate significant differences at $P \leq .001$

** Indicate significant differences at $P < .05$

2.4 Discussion

2.4.1 Reliability- Adults

One of the main aims of the present study was to assess the reliability of a new generation of three types of movement sensing devices, ActiGraph (AG), ActivPAL (AP) and SenseWear (SW). Reliability of AG, AP and SW have been assessed in adults in previous studies and were found to be reliable during walking at a variety of speeds (Wood, 2000; Ryan *et al.*, 2006; Fruin and Rankin, 2004; Gallagher *et al.*, 2007).

The current study supports the previous findings as the results show excellent reliability in both gradients across all speeds (slow walking= 3Km.h^{-1}, normal walking= 4.5Km.h^{-1} and brisk walking= 6Km.h^{-1}) for any of the three accelerometers. The contributions of the present study were assessing the reliability of the new generation of AG model GT1M in estimating EE during walking whereas most of the previous studies assessed the reliability of the AG model MTI 7164 (Manufacturing Technology Inc. 7164) (Wood, 2000).

Moreover, the present study examined the reliability of AP in assessing EE during walking in adults including overweight/obese individuals. SW is more than accelerometer and usually provided with software to analyse the data. Most of the previous studies have evaluated SW reliability using old version such as version 1.0 (Fruin and Rankin, 2004; Gallagher *et al.*, 2007). In the current study, SW data was examined using the most recent version 6.1. In addition, AG, AP and SW showed strong intra-instrument reliability in both gradients as well. Thus, ActiGraph, ActivPAL and SenseWear are dependable

instruments and can be trusted in terms of reliability during different walking speeds including walking on an incline at 5% gradient.

2.4.2 Reliability- Adolescents

Likewise in adults group, the reliability of ActiGraph and SenseWear have been examined previously in adolescents in different studies and have been found to be reliable devices using either previous generation model (AG model 7164) or older software version such as in SW version 1.0 (Trost *et al.*, 2005; Liden *et al.*, 2001). There are no studies evaluating the reliability of AP in assessing EE in apparently healthy adolescents. The contribution of the present study includes an evaluation of the reliability of AG model GT1M in adolescents during walking at different speeds and examined the reliability of the SW using a recent software version 6.1 to analyse the data obtained from adolescents. The current study found that the new model of ActiGraph GT1M, ActivPAL and SenseWear using software version 6.1 were all reliable in assessing EE obtained from adolescents during walking on both gradients.

Furthermore, all accelerometers showed strong intra-instrument reliability between the two trials on both gradients across all speeds. According, these types of instruments are dependable in terms of reliability and can be used to assess different walking speeds on the flat or on an incline (5% gradient) in adults (lean and overweight/obese) and adolescents.

2.4.3 Validity - Adults

Having established that reliability is good, measuring accuracy of accelerometers that have been used to assess EE becomes important. The second main aim of the present study was to assess the accuracy of the new generation of three types of accelerometers, ActiGraph (AG), ActivPAL (AP) and SenseWear (SW).

In contrast with reliability, the data illustrated that these accelerometers all had problems with validity during walking speeds on the flat and at 5% gradient. In terms of accuracy of assessing activity EE (Kcals.min^{-1}), AP was the poorest device of the three devices during all walking speeds. Although intraclass correlation coefficient (ICC) showed moderate to high correlation between AP and indirect calorimetry values in most occasions, the T-tests output demonstrated that AP was not accurate enough in any of walking speeds on both gradients for both groups, except in lean group on the flat at normal walking speed (4.5 Km.h^{-1}). Furthermore, AP was not sensitive enough to detect activity EE differences between walking speeds in overweight/obese group on both gradients.

Most of the studies that have used AP in adults measured the time spent in static activities (sitting/lying and standing) and dynamic activity (stepping and cadence (steps.min^{-1})) (Dahele *et al.*, 2007; Godfrey *et al.*, 2006; Koulouri *et al.*, 2006; Godfrey *et al.*, 2008). AP was found to be reliable and valid in terms of assessing step number and cadence during walking at four different speeds (between 3.24–6.4km.h^{-1}) (Ryan *et al.*, 2006). In healthy adults, about 65±5% of total physical activity EE is generated from resting and sedentary lifestyle

and about 35±5% of EE comes from physical activities (varying between light and vigorous) (Dahele *et al.*, 2007). One of the previous studies recommended that validation studies comparing direct physical activity estimated by AP with a gold standard measurement are required (Dahele *et al.*, 2007). The AP accelerometer is calibrated by the company and cannot be recalibrated before being utilised. One of the measurements that can be obtained from AP is the assessment of general physical activity level in EE (METs). The EE that can be obtained from AP is based on the default values of (METs) (1.25 METs for sitting/lying, 1.4 METs for standing and 4 METs for stepping at a cadence of 120 steps per minute (brisk walking pace)) and physical activity EE can be calculated according to the formula provided with the device by the manufacturers (Ainsworth *et al.*, 2000):

$$EE\ (MET.h^{-1}) = (1.4 \times \mathbf{d}) + (4 - 1.4) \times (\mathbf{c}.120^{-1}) \times \mathbf{d}$$

c= cadence (steps per minutes), **d**= activity duration (hours)

This formula is used to assess general physical activity level (METs) which may be suitable for lean healthy adults. However, the results of the present study have demonstrated that AP is not valid to assess activity EE ($Kcals.min^{-1}$) when examining walking at different speeds on the flat and on an incline treadmill, especially in overweight/obese adults.

Data from SW showed moderate to high correlation (using ICC, r (CI)) in most occasions comparing with indirect calorimetry. Yet, T-test analysis clearly indicated that SW was invalid to assess EE ($Kcals.min^{-1}$) on most occasions in the current study design. Particularly, using software version 6.1, SW in the

current study overestimated EE during walking on the flat and underestimated EE at 5% gradients on most occasions regardless of body mass. However, it was more accurate compared to the AP as SW was at least able to accurately assess activity EE during brisk walking (6Km.h^{-1}) in the lean group on the flat as well as during slow walking (3Km.h^{-1}) on a 5% incline for both groups. In addition, SW was sensitive enough to detect EE differences between all speeds for both groups in both gradients. Unlike AP, SW includes a 2-axis accelerometer, heat flux sensor, galvanic skin responses sensor, skin temperature sensor, and near-body ambient temperature sensor (Liden *et al.*, 2001). In addition, SW assesses activity EE based on given information about the user such as age, gender, height and weight. In a previous study, Fruin and Rankin (2004) compared EE (Kcal.min^{-1}) assessed by SW using InnerView^{TM} version 1.0 (BodyMedia, Inc.) against indirect calorimetry during walking on a level treadmill and at a 5% gradient. Their results demonstrated that SW significantly overestimated EE (Kcals.min^{-1}) in lean adults (25.2±3.2yr) comparing to indirect calorimetry during walking on a treadmill (flat) by 38% at 80.5 m.min^{-1} (4.83km.h^{-1}) and by 14% at 107.3 m.min^{-1} (6.44km.h^{-1}) (P=0.02) and underestimated EE during walking at 107.3 m.min^{-1} on a 5% gradient by 22%, (P=0.01) (Fruin and Rankin, 2004). Similar results were found in lean adults in the present study as SW overestimated EE during normal walking at 4.5Km.h^{1} on the flat comparing to calorimetry by 17.5% and underestimated EE during brisk walking (6km.h^{-1}) at 5% gradient by 23.3%. Interestingly, the results of the present study were obtained from using the most recent software version 6.1 produced by the same manufacturer (BodyMedia Inc.) and suggest that the new software algorithms are no better at providing estimates of EE

during incline walking. However, in terms of validity of assessing EE, the new software version 6.1 was relatively more accurate compared to the old version 1.0 as the present study showed that there was no significant difference between EE obtained from SW (using version 6.1) and EE measured by calorimetry during brisk walking at 6km.h^{-1} in lean adults. Additionally, there is a reduction in the overestimation percentage of EE during normal walking 4.5km.h^{-1} (from 38% in version 1.0 to 17.5% in version 6.1). Although there is a noticeable improvement observed in the SW's software between version 1.0 and 6.1, the SW device still has some problems to assess activity EE in adults during walking especially on uphill surfaces. Thus, more research is needed in order to improve the algorithms for the software to be able to estimate EE precisely especially during walking on different gradients.

The third accelerometer used in the present study was ActiGraph (AG). AG is one of the most commonly used accelerometers for assessing free living physical activity (PA) (Crouter *et al.*, 2006a). One of the popular equations used to obtain EE (Kcals.min^{-1}) from AG is Freedson's equation for lean adults (Freedson *et al.*, 1998). Freedson developed the regression equation on a sample of 35 lean adults (23.8$\pm$4yrs) including males and females. Participants performed 2 walking speeds (4.8 and 6.4Km.h^{-1}) and 1 running (9.7Km.h^{-1}) speed on a treadmill. The equation was subsequently cross validated on a sample of 15 participants. Across all speeds the data showed excellent correlation between actual and predicted EE from AG using the developed equation ($r=0.93$, SEE= $\pm$0.93kcal.min^{-1}, $p<0.05$).

The contribution of the present study was using one of the most common equations (Freedson's equation) to predict activity EE for lean and overweight/obese adults during different walking speeds on the flat and at 5% incline. Results of the current study showed good to high correlation with calorimetry in each speed separately for both groups on both gradients (range between r=0.60 – 0.92). However, the ability of AG to assess EE during different walking speeds on the flat and at 5% gradient was limited. The obtained results from paired T-tests showed that AG was valid to assess EE (Kcals.min^{-1}) only during normal walking (4.5Km.h^{-1}) and brisk walking (6Km.h^{-1}) speeds on the flat for the lean group as well as during normal walking (4.5Km.h^{-1}) for the overweight/obese group at 5% gradient. On the other hand, AG underestimated the EE in lean during slow walking (3Km.h^{-1}) on the flat and during all speeds on an incline. In addition, AG also, underestimated EE in the overweight/obese group during brisk walking (6Km.h^{-1}) on an incline. In contrast, AG overestimated EE in overweight/obese group during all walking speeds on the flat as well as during slow walking on an incline.

These results concur with the study by Freedson *et al.* (1998); as both studies confirm the ability of AG in assessing EE for lean adults during walking speed between 4.5 – 6.4 km.h^{-1} on flat surfaces using Freedson's equation. The results of the present study partly concur with Slootmaker *et al.* (2009) in their recent study. They investigated the validity of AG in lean adults during walking on a treadmill and walking up and down stairs. The results indicated that AG underestimated EE using Freedson's equation during walking speed of 3km.h^{-1} on the flat by 44% and by more than half of the measured EE using calorimetry

(>50%) during walking the stairs at 80 and 100 steps per minute. (Slootmaker *et al.*, 2009).

The number of studies that have examined the ability of AG to estimate EE and/or physical activity has increased in the last decade (Troiano RP. 2005; Freedson *et al.*, 1998; Hendelman *et al.*, 2000; Crouter *et al.*, 2006a; Crouter *et al.*, 2006b). Several studies have examined the accuracy of AG in detecting EE comparing to indirect calorimetry for adults during various physical activities in the lab and/or in the field for lean healthy adults (Matthew, 2005; Hendelman *et al.*, 2000; Crouter *et al.*, 2006a; Crouter *et al.*, 2006b). Some of these studies have shown that the EE estimated by AG is affected by the intensity of the workload used (Matthew, 2005; Crouter *et al.*, 2006b). For example, in previous research, Hendelman *et al.* (2000) examined the validity of AG in lean adults and concluded that AG underestimated EE during light activities such as household activities or during upper body movement, when carrying loads, or with changes in surface or ground. On the other hand, Matthew *et al.* (2005) has reviewed a number of studies that evaluate AG accelerometer during different physical activity including walking and running. They concluded that counts of AG increased with increase in ambulatory speeds which indicates that AG is a strong objective measure for dynamic physical activity. Although some physical activities are unlikely to be accurately measured by AG such as highly static exercise or weight-lifting, AG is an adequate instrument to assess physical activity levels and intensity during habitual physical activity (Matthew, 2005). Moreover, the AG was found to be the most accurate in assessing EE during moderate activity such as walking (Freedson *et al.*, 1998; Hendelman *et*

al., 2000; Crouter *et al.*, 2006b). Therefore, the Freedson's equation used for AG to predict EE needs development so that it can provide acceptable estimation of EE during different types of physical activity such as walking, jogging and running and to make it suitable for lean and overweight/obese adults, especially if any useful energy intake vs. energy expenditure comparisons are to be made that would add real value to this cheap objective measure of physical activity.

Moreover, multiple comparison one-way ANOVA was used to examine the ability of AG to detect EE differences between speeds. The results showed that AG detected EE differences between all speeds for both groups in both gradients. In addition, AG was better than AP and SW in terms of detecting EE differences while changing surface (flat & 5% gradient) in all speeds for both groups. In contrast AP was found to be insufficiently sensitive to detect EE differences while changing in surface level (flat to 5% incline) during slow ($3Km.h^{-1}$) or normal ($4.5Km.h^{-1}$) walking speeds for both groups. Similarly, SW was not able to detect EE differences while changing in surface level during slow and normal walking speeds for lean group only. Thus, AG is considered as the most sensitive device for assessing physical activity during walking on flat surfaces.

Although all accelerometers have limitations, AG seems to be the most appropriate device to assess EE and physical activity for adults comparing to the other devices (SW and AP).

2.4.4 Validity - Adolescents

Part of the purpose of this study was to assess the validity of the three different accelerometer devices (ActiGraph AG, ActivPAL AP and SenseWear SW). Data from the accelerometers was compared with indirect calorimetry gas analysis using Intraclass Coefficient Correlation (ICC) and 95% confidence interval (CI). Although, data showed high correlation between AP and calorimetry in both gradients (on flat r= between 0.81 – 0.83, at 5% grade r= between 0.73 – 0.83), $p<0.001$, AP did not appear to be statistically (examined by paired samples t-test) valid in terms of assessing EE ($kcals.min^{-1}$) for adolescents during walking speeds in both gradients except during slow walking at $3km.h^{-1}$ on flat surface.

This device may not be suitable to assess EE for adolescents during free-living activity especially if the activities include moderate to vigorous physical activity (MVPA). Data in the current study clearly showed that AP underestimated EE during all speeds in both gradients except at slow walking on the flat. In terms of physiological response, energy cost in adolescents is changeable as maturation occurs and also adolescents have higher energy cost comparing to adults (Freedson *et al.*, 2005). Thus, it is important to take this point into account when using AP to assess EE in adolescent populations especially if they engage in a high level of physical activity or during physical exercise on anything other than flat surfaces. AP has been found to be reliable and valid in terms of assessing steps number and cadence during walking (Ryan *et al.*, 2006). Most studies that use AP were on adults and were measuring steps and cadence ($steps.min^{-1}$) as well as the time spent in static activities (sitting/lying

and standing) and dynamic activity (stepping and cadence) (Dahele *et al.*, 2007; Godfrey *et al.*, 2006; Koulouri *et al.*, 2006; Godfrey *et al.*, 2008). There is lack of research on adolescents using AP. Ryan *et al.* (2006) concluded that the ActivPAL is an effective device for measuring cadence and step number in healthy adults. However, further research is needed to determine if it will also be useful in populations with an obviously different gait, such as children or the elderly (Ryan *et al.*, 2006). Furthermore, there is no published study before 2009 found in literature that has examined the accuracy of AP on healthy children/adolescents. One of the most recent studies by Godfrey *et al.* (2008) mentioned that "The monitor (ActivPAL) also provides data for energy expenditure (METs.hour, Physical Activity Level (PAL), kCAL) that is derived from the activity parameters; however, these have not been independently validated". In addition, AP showed unacceptable sensitivity as it could not detect EE differences between walking speeds in adolescents on both gradients. Thus, more research on adolescents population are needed to improve the accuracy of the AP.

On the other hand, AG showed significant high to very high correlation with indirect calorimetry (IC) when combining all speeds in both gradients (r= between 0.84 – 0.95) and moderate to high correlation in other speeds (4.5Km.h^{-1} and 6Km.h^{-1}) on the flat and at 5% uphill surface (r= between 0.62 – 0.85) respectively all at P<0.05. In the present study, the results showed that AG using Freedson's equation was not accurate in assessing EE during walking even on the flat. Comparing EE obtained from AG (Kcal.min^{-1}) to calorimetry indicated that AG significantly underestimated EE during walking

(range by 14.8% - 71.8%) in both gradients. The equation used to obtain EE (Kcal.min^{-1}) from AG GT1M could not estimate EE accurately in adolescents during walking in both gradients. Therefore, caution should be taken when applying Freedson's equation for adults on adolescent population even if the adolescents have significantly similar predicting VO_{2max} (ml.kg^{-1}.min^{-1}). Bassett *et al.* (2000) concluded that "no single regression equation appears to accurately predict energy expenditure based on acceleration score for all activities". Until appropriate methods of data collection, processing and interpretation of AG outcomes established to standardise using of AG, caution should be taken when using published equation for AG counts to assess PAEE (Freedson *et al.*, 2005).

In adolescent, however, AG GT1M was sensitive enough to detect EE differences between all examined walking speeds for adolescents in both gradients. Thus, AG is a valid to detect even a slight change in physical activity intensity performed by adolescents.

In contrast, EE estimated by SW using software version 6,1 was found to be significantly correlated to calorimetry across all speeds in adolescents as a group, and in each gender separately in both gradients. Although a high correlation was found between SW and calorimetry in brisk walking (6Km.h^{-1}) on a flat surface and at 5% gradient, this device overestimated EE at slow walking speeds (3Km.h^{-1}) by 30% and there was no significant correlation found between SW and indirect calorimetry on the flat or at 5% incline. Nevertheless, paired samples T-test clearly showed that the mean EE obtained from SW overestimated EE comparing to the value obtained from indirect

calorimetry on most occasions. This result was found in adolescents as a group in both gradients (except at normal walking speed on a 5% grade) and in each gender on the flat. The only cases that SW was valid comparing to IC were measured on an incline (5% grade) at normal speed (4.5Km.h^{-1}) and across all speeds in males and females groups. These findings were obtained from SW using a recent algorithm developed in software version 6.1 provided by the manufacturer SenseWear PRO$_2$ Armband®. Mealey (2008) investigated the validity of SW with adolescents sample (n=20, 10.6±1.23yr) using the same SenseWear Professional software (version 6.1). She found that activity EE obtained from SW is overestimated by 13% during walking on the treadmill at 4.8Km.h^{-1} on the flat (Mealey, 2008).

Another study by Arvidsson and colleagues (2007) investigated the validity of SW in children (11-13yrs) during different types of physical activity including setting and playing games using mobile phone, walking, jogging, running, bicycling, stepping and jumping on a trampoline. The results of that study demonstrated that SW underestimated EE when software version 5.1 was used, and the underestimation of EE increased with increased physical activity intensity (Arvidsson *et al.*, 2007). A recent study by Dorminy *et al.* (2008) examined the accuracy of SW for assessing EE in African American children aged 10–14 years old during treadmill exercise test, sedentary activities, rest, sleep, and total 24-h EE using indirect room calorimetry (IRC) as a standard gold. They found that the algorithms used from version 4.1 to predict EE during these activities from SW may not be applicable to children and adolescents as it is mostly developed for adults (Dorminy *et al.*, 2008). They demonstrated that

SW with using software version 4.1 overestimated EE during the activities performed by children (ranged between 16–43%) (Dorminy *et al.*, 2008). It seems important for the SW manufacturer to take into account these results including the present study to improve the ability of SW to predict EE in children and adolescents during physical activity. It also seems that the multiple sensor arrangement of SW does not improve the validity of the device in assessing activity EE.

The SW was not sensitive in detecting EE differences between speeds. This was observed for males between normal (4.8Km.h^{-1}) and brisk (6Km.h^{-1}) walking speeds on both gradients and in female group between slow (3Km.h^{-1}) and normal (4.8Km.h^{-1}) walking speeds on the flat. The developed software version 6.1 using with SW device to assess EE for adolescents should be investigated in different physical activity such as during free-living activity so that it becomes more precise to detect the small EE differences that excess between physical activities.

Thus, of the three devices they all have issues with validity but the AG device is sensitive enough to detect differences in activity intensity across the speeds examined and therefore it would seem prudent to recommend this device for further work examining activity intensities in free living situations. The other devices (AP and SW) are not sensitive enough to detect meaningful changes in activity energy expenditure and are therefore not suitable for free living activity studies that are interested in monitoring intensities of activity.

2.4.5 Heart rate measurement and ActiGraph – Adults

Heart rate (HR) measured for adults participants during the laboratory exercise test. The result showed that HR was significantly different between all speeds for both lean and overweight/obese groups in both gradients. On the other hand, AG in this study showed ability to detect the predicted EE differences between speeds in adult participants. However, AG was not significantly valid to assess EE during light activity such as walking at 3 or 4.5 $km.h^{-1}$ on flat surface. The ability of combining HR and AG output to predict EE was examined in the present study by combining the counts per minute (CPM) of AG with HR ($CPM.b^{-1}$). The $CPM.b^{-1}$ detected the predicted EE differences between speeds for both adult groups in both gradients and the correlation between $CPM.b^{-1}$ and indirect calorimetry (IC) was high on the flat for both groups. However the correlation was low on an incline for both groups. Thus, the combination of HR and AG may provide useful to estimate EE in adults regardless of body weight during light activity such as walking on the flat.

2.4.6 Heart rate measurement and ActiGraph – Adolescents

All adolescents as a group showed significant differences in heart rate (HR) between speeds in both gradients. Also, HR measurement in male and female groups separately indicated significant differences between all speeds at 5% gradient. However, on the flat, both male and female groups showed insignificant differences between slow walking ($3Km.h^{-1}$) and normal walking ($4.5\ Km.h^{-1}$) speeds. One explanation could be that all of the adolescent

participants were lean and most of them were fairly active (MVPA ≥300mins.wk^{-1}). Thus, a small change in speeds might not affect the HR to a noticeable degree especially between light to somewhat moderate physical activity in very active adolescents. Although HR monitoring can be used to assess physical activity, HR monitoring alone may not be accurate in assessing EE during free-living activity as HR monitoring has been found to overestimate EE by ≥12% compared with double-labelled water DLW (Emons *et al.*, 1992).

On the other hand, the counts per minute (CPM) of AG were also combined with the HR to assess EE during walking speeds. The combination was done (CPM.b^{-1}) for each workload for adolescents as a group and for each gender separately in both gradients. The mean (SD) and mean differences CPM.b^{-1} detected the EE differences between speeds for all adolescents as a group and for each gender separately on both gradients (except in females on an incline between normal and brisk walking speeds). Yet, the correlation between CPM.b^{-1} and indirect calorimetry was low in adolescents as a group and in each gender separately on both gradients. The recorded ICC was between r(CI)= 0.159(-0.53 – 0.54) and r(CI)=0.357(-0.20 – 0.66) and all at *P*>0.001. Therefore, results in this study demonstrated that a combination of HR with AG (count/min) could not serve to better predict EE during walking in children/adolescents. Further studies are needed to improve a suitable equation to detect EE during free-living activity preformed by children/adolescents.

2.5 Conclusion:

The main aims of the present study were to examine the reliability and validity of ActiGraph (AG), ActivPAL (AP) and SenseWear (SW) in estimating EE against indirect calorimetry during walking at different speeds on the flat and on an incline (5%) in adults and adolescents using experimental laboratory work.

The present study demonstrates that the new generation of these devices (AG, AP and SW) were reliable on the flat and at 5% gradient for lean and overweight/obese groups and adolescents across all walking speeds. In addition, all accelerometry devices showed strong intra-instrument reliability across all speeds for both adults groups and adolescents in both gradients.

Validity of these devices was assessed in both adults and adolescents. In adult participants, AG GT1M and SW (using software version 6.1) were valid in estimating EE during walking on more occasions than AP. The AG GT1M was able to accurately assess EE during normal ($4.5Km.h^{-1}$) and brisk ($6Km.h^{-1}$) walking speeds on the flat in lean adults as well as at normal walking speed ($4.5Km.h^{-1}$) on a 5% incline in overweight/obese adults. The SW showed ability to estimate EE during brisk waking ($6Km.h^{-1}$) in lean on the flat and during slow waking ($3Km.h^{-1}$) speed at 5% incline in both groups. The AP, on the other hand, was not able to assess EE during walking speeds except at normal walking ($4.5Km.h^{-1}$) in lean adults on flat surface.

The intraclass correlation coefficient (ICC) between each one of these devices (AG GT1M, AP and SW) and indirect calorimetry varied between moderate excellent correlations across all speeds for both groups in both gradients.

The sensitivity of these devices to detect EE differences between walking speeds in adult participants was found in AG and SW more than AP, as AG and SW were able to detect EE differences between walking speeds for both groups in both gradients whereas AP was able to detect EE only between walking slow and fast walking speeds in lean on both gradients.

In adolescents, all three devices had problems with validity in most occasions. On the flat, none of the accelerometers were valid to assess EE during walking speeds in adolescents except AP during walking at 3Km.h^{-1}. On an incline, SW was the only device that could predict EE during normal walking 4.5Km.h^{-1} as well as at a cross all speeds for each gender group.

The correlation (ICC) between the estimated EE by each of these devices and indirect calorimetry data showed that all three devices (AG, AP and SW) showed good to high correlation to the EE obtained from calorimetry across all speeds in both gradients for all adolescents as a group and for each gender separately.

On the other hand, the ability of these devices (AG, AP and SW) to detect EE differences between speeds indicated that AG was sensitive to detect EE differences between all speeds for all adolescents as a group and for each gender separately in both gradients. However, SW could detect EE between speeds in adolescents as a group in both gradients as well as in female group on an incline. In contrast, AP unable to detect the EE differences between speeds in most occasions.

In terms of the combination between HR and AG, the current study demonstrated that such a combination may provide useful estimation of EE in adults regardless of body weight during light activity such as walking on the flat. Nevertheless, this study demonstrated that a combination of HR with AG could not estimate EE during walking in adolescents. Further studies are needed to improve a suitable equation to detect EE during free-living activity preformed by adolescents.

In general, these accelerometers were reliable for both adults and adolescents during walking in different speeds. However, more studies are requested in order to improve the ability of these devices to accurately estimate activity EE during one of the most common physical activity such as walking in different speeds. Of the devices the overall most sensitive device for detecting differences in activity intensity in all participants was the AG.

Chapter 3: Physical activity assessment of lean and overweight/obese adults using accelerometry and heart rate monitoring.

3.1 Introduction

Accurate assessment of habitual free living physical activity is clearly very important since being physically inactive may lead to several types of disease such as cardiovascular disease (Panagiotakos *et al.*, 2003; Fang *et al.*, 2003), osteoporosis (Kai *et al.*, 2003) and type II diabetes (Kriska *et al.*, 2003; Hu *et al.*, 2003). Over the last few decades the prevalence of overweight and obesity has increased dramatically (King *et al.*, 2004), and this is partly related to low physical activity status (Jebb and Moore, 1999). Habitual physical activity can be assessed by different subjective instruments such as self-recording, questionnaires, or physical activity recall. Although these instruments are inexpensive and easy to control, adult participants often overestimate their activity. In some studies, obese participants have been found to overestimate their physical activity by 30% to 50% (Lightman *et al.*, 1992; Jakicic *et al.*, 1998) and in another study, lean adults have been found to overestimate physical activity by 8% to 30% (Conway *et al.*, 2002). Therefore, objective measures or physiological markers may provide a more accurate assessment of physical activity in terms of time spent in activity and the distribution of activity intensities.

In the last decade, the number of studies that have used accelerometry as an objective measure to assess habitual physical activity (time spent in each physical activity level or physical activity energy expenditure) has increased

dramatically (Freedson *et al.*, 1998; Plasqui and Westerterp, 2007; Crouter *et al.*, 2006b; Kwak *et al.*, 2007; Hendelman *et al.*, 2000). Some of these studies have aimed to estimate physical activity energy expenditure (PAEE) using AG (Crouter *et al.*, 2006a; Plasqui and Westerterp, 2007; Crouter *et al.*, 2006b), whereas others have used AG to assess habitual physical activity purely on intensity classifications for physical activity levels (i.e. Sedentary, Light, Moderate, Vigorous and Very Vigorous) (Kwak *et al.*, 2007; Hendelman *et al.*, 2000; Davis *et al.*, 2006). Some studies have tried to improve the accuracy of AG by combining HR monitoring with accelerometry (Johansson *et al.*, 2006). However, a number of fundamental factors that influence the accuracy of accelerometry (i.e. Actigraph) for assessing physical activity or PAEE have been discussed in a recent conference (Troiano, 2005).

The validity and reliability of AG in assessing physical activity in field-based studies has been examined (Mâsse *et al.*, 2005), and others have investigated the best practices and research recommendations in order to optimise the accuracy of AG (Ward *et al.*, 2005). Although AG has been found to be inaccurate in terms of estimating energy expenditure in different intensity categories during free-living activity, AG has been found as a valid instrument in assessing time spent in different physical activity intensity levels (Welk *et al.*, 2000). Furthermore, the data collected presented in Chapter 2 highlight that all devices have issues with validity but that AG is most sensitive to detecting changes in activity energy expenditure through a range of walking speeds encountered during day to day living.

In terms of intensity, physical activity status has typically been classified into levels such as sedentary, light, moderate, vigorous, and very vigorous. In addition to the intensity, physical activity assessment includes, time spent in the activity (duration), and number of activity sessions (frequency) (Caspersen *et al.*, 1985). The intensity, duration and frequency of recommended aerobic exercise may vary according to the health purpose. In 1995, the Centres for Disease Control (CDC) and the American College of Sports and Medicine (ACSM) established physical activity recommendations for healthy adults for health benefits (Pate *et al.*, 1995). They recommended 30 minutes or more of moderate intensity physical activity per day, preferably all days of the week (Pate *et al.*, 1995).

In 2007, ACSM and American Heart Association (AHA) updated the recommendations for healthy adults to promote and maintain health to be aerobic physical activity for a minimum 30 minutes of moderate intensity on five days per week or vigorous intensity of 20 minutes or more on three days per week. A combination of moderate and vigorous intensity of aerobic physical activity can be performed to meet this recommendation (Haskell *et al.*, 2007). Similar recommendations were published by Scotland Health Survey (SHeS) and Health Education Population Survey (HEPS) and the Department of Health and Human Services (DHHS) (Catto *et al.*, 2009; U.S. Department of Health and Human Services, 2008).

Interestingly, the DHHS recommended at least 300 minutes (5 hours) of moderate-intensity, or 150 minutes (2.5 hours) of vigorous-intensity per week, or an equivalent combination of moderate and vigorous intensity aerobic

physical activity for gaining additional health benefits and for weight loss purposes (U.S. Department of Health and Human Services, 2008).

The availability of the reliable and sensitive new generation of accelerometer (AG) combined with the updated physical activity recommendations for apparently healthy adults allows a more detailed assessment of the physical activity status in adults. In some studies, obese adults have been found to spend significantly less time in moderate physical activity compared to the lean (Ekelund *et al.*, 2002). However, more than one study found that there were no significant differences in activity counts (count per minute) between obese and non-obese adults (Tyron, 1987; Meijer *et al.*, 1992). In a UK study, the differences between obese and non-obese adults in time spent in recommended physical activity have been found only on weekends but not on weekdays (Cooper *et al.*, 2000).

There are few studies in the Scottish population that have used the new generation of AG as an objective physical activity measure to compare the habitual physical activity of healthy lean versus overweight/obese adults and to evaluate their physical activity status in relation to the updated physical activity recommendations.

3.1.1 Aims of the study

To assess the habitual physical activity of healthy lean and overweight/obese adults using a new generation of AG (GT1M) as well as HR monitoring and to

compare the habitual physical activity between groups of adults in the Scottish population.

To assess the agreement between physical activity status of the research group studied with the recommended guidelines published by ACSM (2007), SHeS & HEPS (2008) and DHHS (2005) for adults.

3.2 Methodology

3.2.1 Subjects

A total of 60 healthy adults aged 18 -55 years old were recruited to take part in this study after successful approval for the study was obtained from the research ethics committee of University of Stirling and from the local NHS research ethics committee. 57 of the total participants completed their participation in this study and 3 of them withdrew for personal reasons. Participants were divided into two groups based on body composition: 34 lean (males n=16, females n=18) and 23 overweight/obese (males n=12, females n=11). Physical characteristics of the participants are indicated in Table 3.1.

Table 3.1 Physical characteristics expressed as means ±SD for 57 adults, (lean n=34, overweight/obese n= 23)

Physical characteristics	**Lean** **n= 34**	**Overweight/Obese** **n= 23**
Age (yrs)	35.7±10.6	40.3±8.4
Mass (Kg)	67.2 ±9.2	90.6 ±13.0 *
Height (cm)	169.2 ±9.47	173.6 ±8.5
BMI ($Kg.m^{-2}$)	23.3 ±1.8	30.1 ±3.9 *
Body Fat%	22.0 ±7.2	35.5 ±12.1 *
Fat Free Mass (Kg)	52.2 ±9.6	57.4 ±12.3
Mean arterial pressure (MAP)	86.8±8.7	96.1±8.2 *
Resting heart rate (bpm)	65.4 ±9.3	70.9 ±10.4 *
Predicted VO_{2max} ($ml.kg^{-1}.min^{-1}$)	48.1 +10.5	32.8+6.3 **

* indicates a significant difference between groups at P< 0.05.

** indicates a significant difference between groups at P<0.01.

All participants were recruited from the public people in Stirlingshire council area including University of Stirling staff and students. All participants were informed about the study by an invitation letter, poster and/or leaflet. Subsequently, interested participants who would like to take part in the study contacted the researchers via email, letter or telephone. Then they were provided with an information sheet describing the study in detail. If they decided to take part in the study, an initial briefing visit (first visit) was arranged with them to explain all parts and stages of the study.

3.2.2 Study design

3.2.2.1 Anthropometric and body composition

Participants attended the research science laboratory at the University of Stirling on two occasions. During the first visit, participants were given an explanation of the procedures of the study including how to use the devices (Actigraph, and heart rate monitor). Figure 3.1 illustrates how they were instructed to wear the devices and if they had any questions these were answered. If they were still happy to participate they signed a consent form which was constructed by the principal investigator. After that, the following body measurements were taken: blood pressure, resting heart rate, weight, height, and body composition.

Body mass was measured in Kilograms to the nearest 50 grams and height was measured in Centimetres to the nearest 1 millimetre. Based on the calculated body mass index (BMI) (body mass (Kg)/(height (m))^2) and estimated body fat (BF) percentage (using foot to foot impedance analysis), participants were classified into one of the groups (lean or overweight/obese). The classification method was provided by ACSM's guidelines (American College of Sports Medicine, 2005). Thus, the participants were classified as follows:

Male: Lean if BMI <25 or BMI ≥25 and BF < 20% for (18-39yrs)

BF < 22% for (40-59yrs)

Overweight/Obese if BMI ≥ 25 and BF > 20% for (18-39yrs)

BF > 22% for (40-59yrs)

Female: Lean if BMI <25 or BMI > 25 and BF < 33% for (18-39yrs)

BF < 34% for (40-59yrs)

Overweight/Obese if BMI ≥25 and BF > 33% for (18-39yrs)

BF > 34% for (18-39yrs)

3.2.2.2 Instruments and physical activity

Details of the instruments used for this study are found in Table 3.2.

Table 3-2 name and details of instruments used for physical measurements.

Measurements	**Name and details of the instrument**
Impedance analysis (Body Fat %)	TANITA, Model: TBF-105, TANITA UK Ltd., Made in USA
Blood pressure & resting HR	OMRON, Digital Blood Pressure Monitor OMRON Electronics Ltd, Model: HEM-405C
Body mass	John White & Son, (Weighing Machines) Ltd., Made in Scotland
Height	Holtain Ltd., Made in Britain

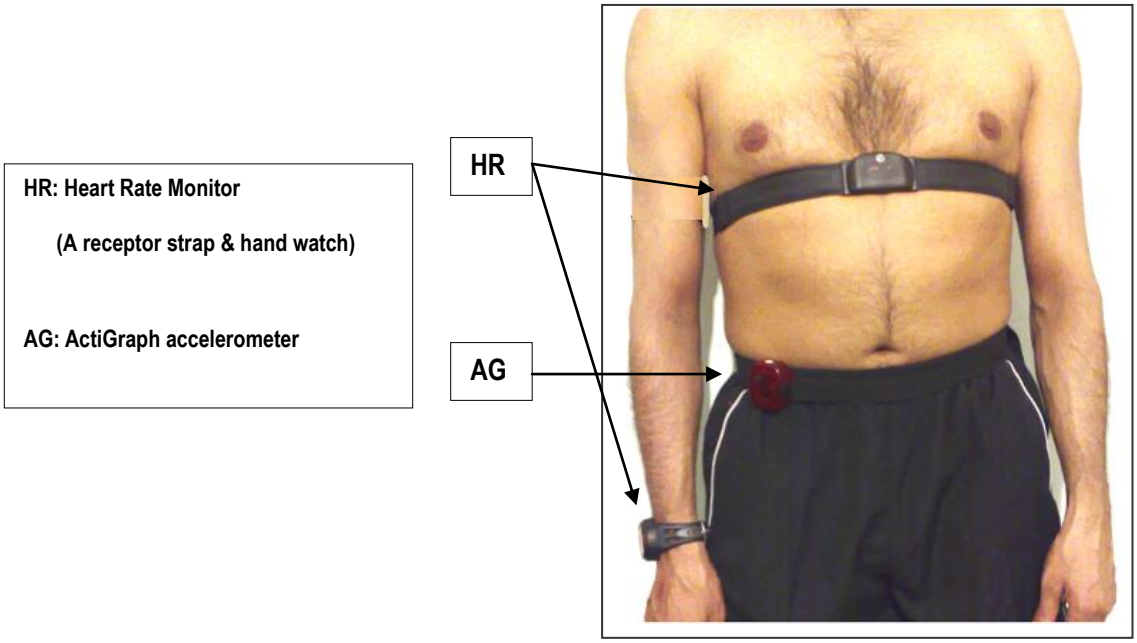


Figure 3.1 Illustration of how the accelerometer (AG) and hear rate monitor (HR) are put on the body.

During the initial visit, both AG and HR were set up for the participant based on his/her characteristics and a code number was recorded. AG was set to record in counts per 15 second and steps count was activated. Then participants were asked to wear the devices for two blocks of six days of physical activity monitoring. In each block of six days, participants were instructed to wear both devices as shown in Figure 3.1. Furthermore, they were asked to wear both devices at the same time and to take them off at the same time and to wear them for at least 10 hours of waking time (usually from morning until evening). In addition, they were provided with a physical activity log (PA log) (Appendix C). The PA log was to record moderate physical activity or more. The moderate, vigorous and very vigorous PA intensities were described with some examples. The PA log was given to provide data about their physical activity if

the AG and HR monitor were removed during physical activity involving direct contact activities such as Judo, or performing water based activities such as swimming, as both devices are not considered waterproof. The other purpose of the PA log is that they were asked to record the time of the day when they attached the devices on the body or removed them from the body. For AG, they were not asked to do anything with the device other than attach it to their body and remove it from the body for every measured day. Because the heart rate monitor needs to be started and stopped each time it is worn, the participants were provided with an instruction leaflet of how to attach and use the HR monitor (Appendix C). All instruction leaflets, PA log, information sheet as well as the two devices were provided to each participant in a plastic carrier file.

On the second visit, each participant was asked to walk on a treadmill (on the flat) in 3 different speeds ($3km.h^{-1}$, $4.5km.h^{-1}$ and $6km.h^{-1}$) and oxygen consumption was calculated using indirect calorimetry. This exercise test was used to calculate a predicted VO_{2max} and to determine MET for each individual participant during the three different intensities with the HR data to obtain an individual linear regression equation for each participant in order to evaluate the assessment of free-living physical activity. Following this visit the participants then completed the second block of 6 days of free living activity monitoring. All participants were provided with contact details of the principal researcher to contact them when they had any problem. At the end of each six day period, each participant was asked to attend to the lab and return the plastic files including the devices as well as the logs. Data from AG and HR were

downloaded to the computer and saved in MS Excel files. Then the devices were charged and re-installation ready for future data collection.

3.2.2.3 Data analysis and statistics

Data obtained from AG were processed and analysed two times by a custom-written programme MAHUffe.exe available from (www.mrc-epid.cam.ac.uk). Results obtained from MAHUffe software programme were checked by comparing these results to the results of a manual calculation method in MS Excel using some of the participant data and both results were identical (Appendix D).

Physical activity data was analysed twice. The first was in 1 minute bout duration and the second was in 10 minute bouts duration. Both analyses were done using the feature in the custom-written programme MAHUffe that allows justifying the bout duration of the registered physical activity data (in the “ContT Criteria” section).

In the first analysis, 1 minute bout duration was used in order to calculate all times of physical activity ($\geq$1min). Activity data were cleared from periods when AG was not worn by excluding consecutive strings of zeros lasting 20 minutes or more (Freedson *et al.*, 1998). All days of less than 10 hours of recorded data were excluded (Freedson *et al.*, 1998; Ward *et al.*, 2005). Participants who had less than 3 days of acceptable data were excluded from the group (Ward *et al.*, 2005). Also the main variable from AG was the average intensity of PA (counts.min^{-1}). Freedson’s cut-points for assessing habitual physical activity for

adults was used for classifying PA into sedentary (<100 counts.min^{-1}), light (100-1952 counts.min^{-1}), moderate (1952-5724 counts.min^{-1}), vigorous (5724-9894 counts.min^{-1}) and very vigorous (>9894 counts.min^{-1}) activity (Freedson *et al.*, 1998). These cut-points were developed by Freedson's and colleagues using the older model of AG (MTI 7164). However, the new model used in the present study (GT1M) has been found to be comparable to MTI 7164 in terms of physical activity intensity classifications during habitual physical activity assessment for adults (Kozey *et al.* 2010). The amount of time (in minutes) spent in each level of PA was calculated as well as the total registered time. All calculated variables were used to compare lean versus overweight/obese adults.

In the second phase of analysis, all procedures were repeated with a change in activity bout time. 10 minutes minimum bout duration was used to calculate only physical activity that lasted 10mins or more. All physical activity with less than 10 minutes was excluded

Both methods were used because both are important to assess habitual physical activity for adults. On the other hand, MVPA in 10 min bouts is thought to have more advantages over MVPA in 1 min bouts in terms of affecting BMI and/or waist circumference in overweight/obese adults (Haskell *et al.*, 2007).

In addition, habitual physical activity was also assessed using HR monitoring (Polar S-625X^{TM}). The assessment of physical activity levels was calculated for each participant using the equation of the linear regression between METs and HR. METs was calculated for each participant from the measured VO_2 in the laboratory test. Then, the linear regression equation between MET and HR was

applied on the measured HR in free-living activity for each participant. Habitual physical activity for each participant was then classified based on HR that was equivalent to sedentary <1.5MET, light ≥1.5-3MET, Moderate ≥3-6MET, vigorous ≥6-9MET and very vigorous >9MET. (Appendix C)

All calculated variables were used to compare lean versus overweight/obese adults. Statistical analyses were conducted using SPSS software version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). An alpha level of 0.05 was used for all analyses to indicate statistical significance. All data is summarised as means ± standard deviation (±SD) as well as percentage of means (±SD) for both groups. Independent-samples T-tests were used to compare the measured variables obtained from AG and HR between lean group versus the overweight/obese group.

3.3 Results

A total of 57 out of 60 healthy participants completed their participation in the present study (3 of them (2males and 1female) withdrew for personal reasons). Participants were classified into two groups based on body composition: 34 lean (males n=16, females n=18) and 23 overweight/obese (males n=12, females n=11).

Almost all physical activity data reported in the PA log was measured by AG and HR. The only exceptions were found in 3 participants as they did not attach the devices during some physical activities. One lean participant reported about an accumulation of 25mins of moderate and 10 min of vigorous intensity during

a football game for leisure. Two participants reported water activities without attaching the devices as they are not waterproof. They were one lean participant who reported 20mins of moderate intensity in one swimming session (moderate freestyle). The other participant was an overweight/obese participant who reported a total of 15-20mins of moderate intensity exercise in two separate swimming sessions per week for leisure (including light and moderate effort). The physical activity compendium was used to estimate the intensity (METs) of these types of activity and was added to each participant physical activity data (Ainsworth *et al.*, 2000). The results with or without these data did not affect the final results in all comparisons between groups.

3.3.1 Habitual physical activity assessed by Actigraph – (1min bout).

The results of the habitual physical activity obtained from AG are summarised in Table 3.3. Data showed that both groups were not significantly different in terms of counts per minutes at any physical activity classification. The only difference between groups was in the mean registered time.

Table 3.3 Mean (±SD) physical activity data of the 57 participants (Lean n=34 and Overweight/obese n=23) using 1min bout method.

PA Parameters	**Lean**	**Overweight/obese**
Number of Days	8.8 ±2.9	7.7 ±2.7
Registered Time (Mins.d^{-1})	770.9 ±61.9	737.9 ±48.0 *
Counts/min	509.1 ±128.3	468.1 ±204.5
Sedentary (Mins.d^{-1})	421.3 ±60.2	402.8 ±86.9
Light (Mins.d^{-1})	295.1 ±59.7	287.3 ±67.3
Moderate (Mins.d^{-1})	46.0 ±17.0	43.2 ±25.0
Moderate to Vigorous (MVPA) (Mins.d^{-1})	53.5 ±18.4	47.5 ±30.3
Vigorous & Very Vigorous (Mins.d^{-1})	9.2 ±10.1	4.6 ±7.4

* Significant differences between groups at $P<0.05$.

In order to normalize the data for comparison, percentage time spent in each physical activity levels was used. Figure 3.2 indicates that there was no significant difference between both lean versus overweight/obese groups in the percentage of registered time spent in sedentary behaviours and active behaviours (lean =54.7±6.6% vs. overweight/obese =54.4±10.2%).

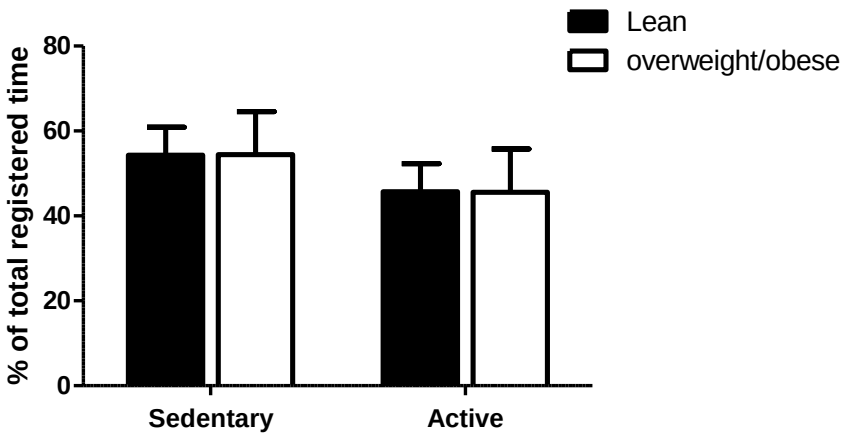
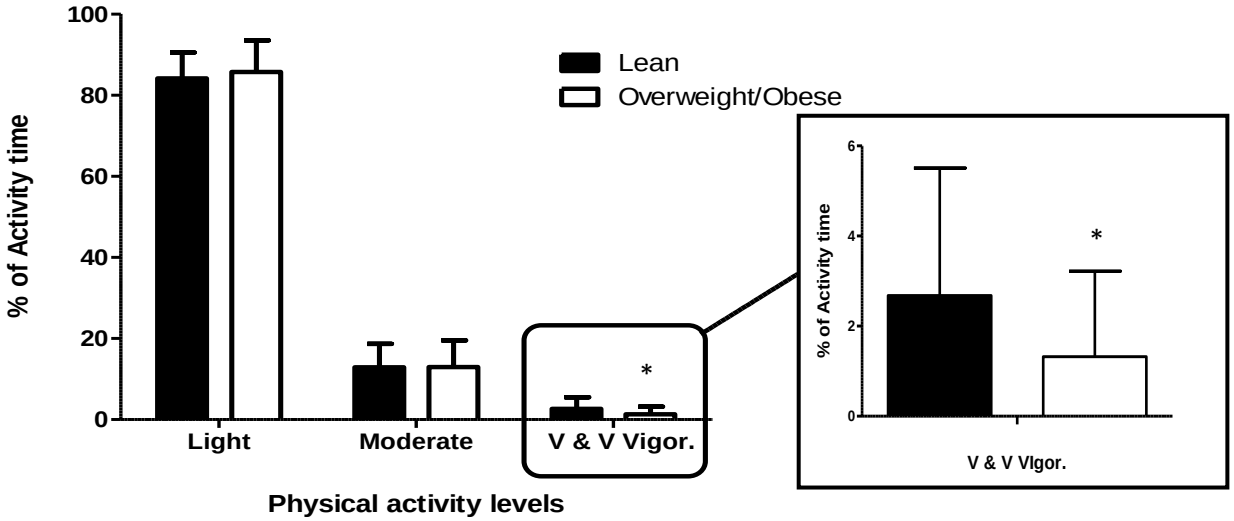


Figure 3.2 Mean(SD) of percentage of registered time spent in sedentary behaviours and active behaviours by 57 adults (lean =34, overweight/obese =23) obtained from Actigraph using 1min bout method.

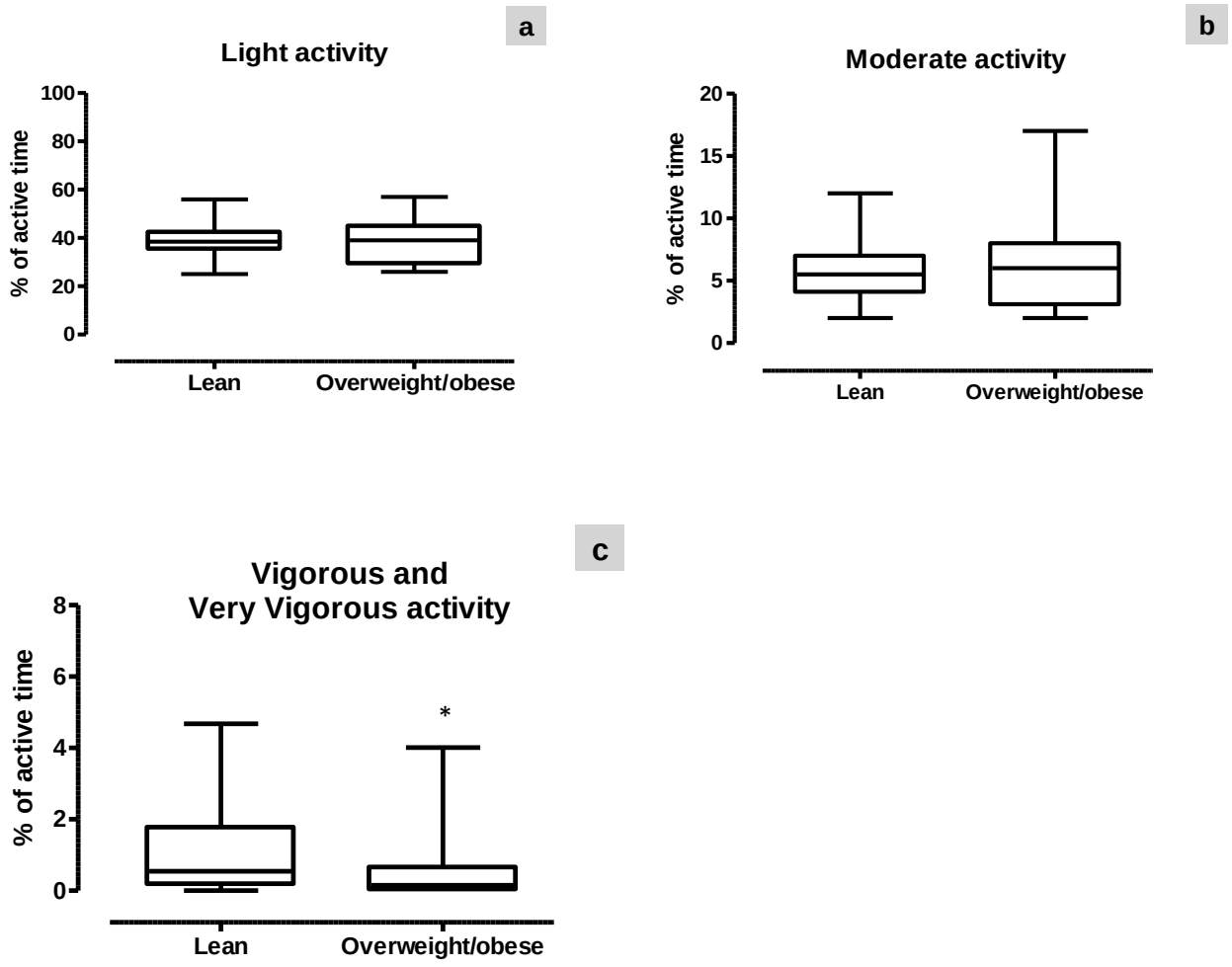
The results of the data obtained from AG using 1min bout shows that the only significant differences between groups were found in percentage of active time spent in vigorous and very vigorous intensity (lean =2.7±2.8% vs. overweight/obese =1.3±1.9% of monitored active time) (Figure 3.3). However, there were no significant differences between groups in percentage of active time spent in light (lean =84.1±6.4% vs. overweight/obese =85.8±7.8% of monitored active time) or moderate activity (lean =12.9±5.8% vs. overweight/obese =12.9±6.6% of monitored active time).



* Significant differences between groups at *P*<0.05.

Figure 3.3 Mean(SD) percentage of registered active time spent in different intensity physical activity by 57 adults (lean=34, overweight/obese =23) obtained from Actigraph using 1min bout method.

However, a box-and-whiskers plot in Figure 3.4 (a, b and c) showed the distribution of data indicating the extreme lower and upper values as well as the middle quartiles of data. Data from Fig. 3.4 showed no obvious differences between groups in percentage of active time spent in light and moderate physical activity levels (Fig. 3.4, a and b). However, the difference between groups was significantly observed in the percentage of active time spent in vigorous and very vigorous physical activity level (Fig. 3.4 c).



* Significant differences between groups at *P*<0.05.

Figure 3.4 A box-and-whiskers plot (a, b and c) represents the percentage of activity time spent in different physical activity between lean (n=34) and overweight/obese (n=23) adults.

Although Table 3.3 showed significant differences in registered time (absolute values) between groups (*P*= 0.04), Figure 3.2 and Figure 3.3 indicate that there is no significant differences between groups in percentage of registered time spent in sedentary and active behaviours (P=0.75, 0.75 respectively). Moreover, data obtained from AG using 1min bout indicates that there were no significant differences found between groups in light and moderate physical activity (P= 0.38 and 0.99 respectively). However, percentage of active time

spent in vigorous and very vigorous activity was significantly higher in lean than overweight/obese adults (*P*=0.04).

3.3.2 Habitual physical activity assessed by Actigraph – (10mins bout).

The data of the habitual physical activity obtained from AG using the 10 min bout method are summarised in Table 3.5. There were three participants (lean=1 and overweight/obese=2) who were excluded during assessment of physical activity using the 10mins bout method because their data fell below the acceptable criteria (all were <3d of at least 10hrs.d^{-1}). The absolute time obtained from AG using 10min bouts showed no significant differences between lean versus overweight/obese groups in all reported data except in percentage of active time spent in vigorous and very vigorous physical activity.

Table 3.4 Physical activity data (mean SD) of the 54 participants (Lean n= 33 and Overweight/obese n=21) 10mins bout method.

PA Parameters	**Lean**	**Overweight/obese**
Number of Days	8.12 ±3.2	6.38 ±2.8
Registered Time (Mins.d^{-1})	763.5 ±72.2	740 ±59.6
Counts/min	521 ±143.7	477.7 ±203.9
Sedentary (Mins.d^{-1})	410.2 ±64.5	395.2 ±85.7
Light (Mins.d^{-1})	297.7 ±62.2	295.2 ±69.2
Moderate (Mins.d^{-1})	46.1 ±18.8	46.0 ±27.7
Moderate to Vigorous (MVPA) (Mins.d^{-1})	54.5 ±21.0	49.4 ±31.6
Vigorous & Very Vigorous (Mins.d^{-1})	9.6 ±10.1	3.6 ±6.0 *

* Indicates that there are significant differences between groups at P< 0.05.

Similar results were found between lean and overweight/obese participants when the 10mins bout method was used. Figure 3.5 shows that no significant differences were found between groups in percentage of registered time spent in sedentary and active behaviours (lean =53.8±7.0% vs. overweight/obese =53.2±9.6%).

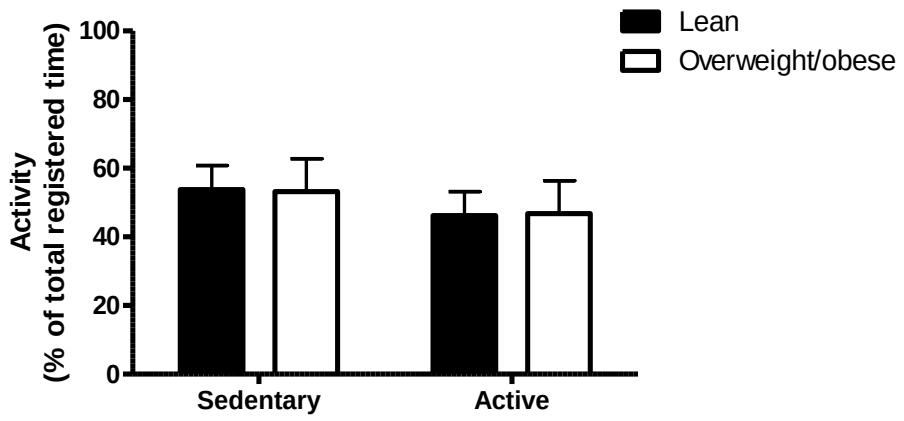
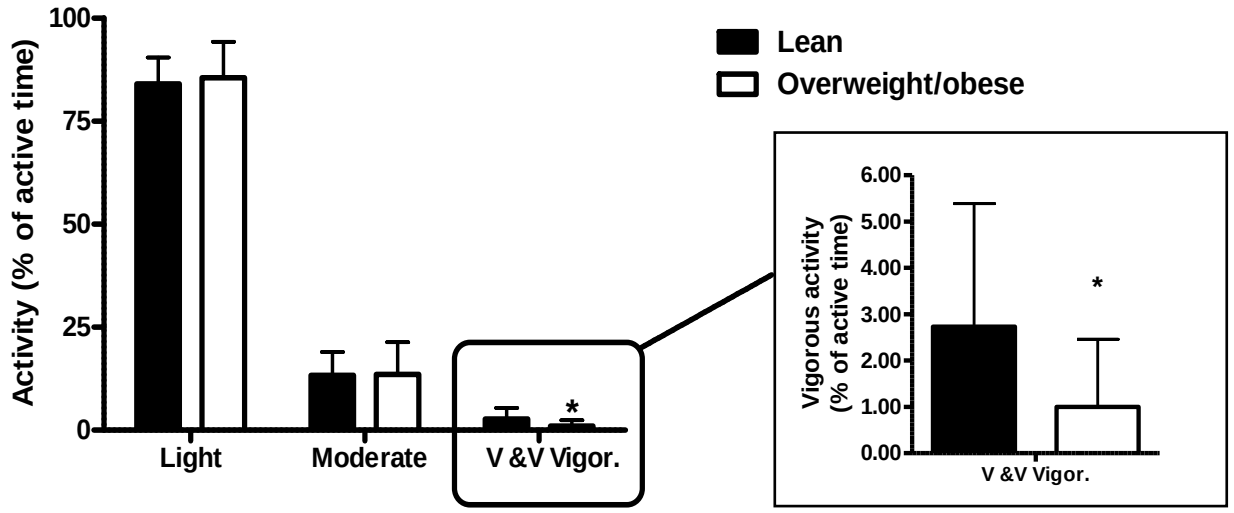


Figure 3.5 Mean(SD), percentage of registered time spent in sedentary and activity time (lean=33, overweight/obese =21) adults.

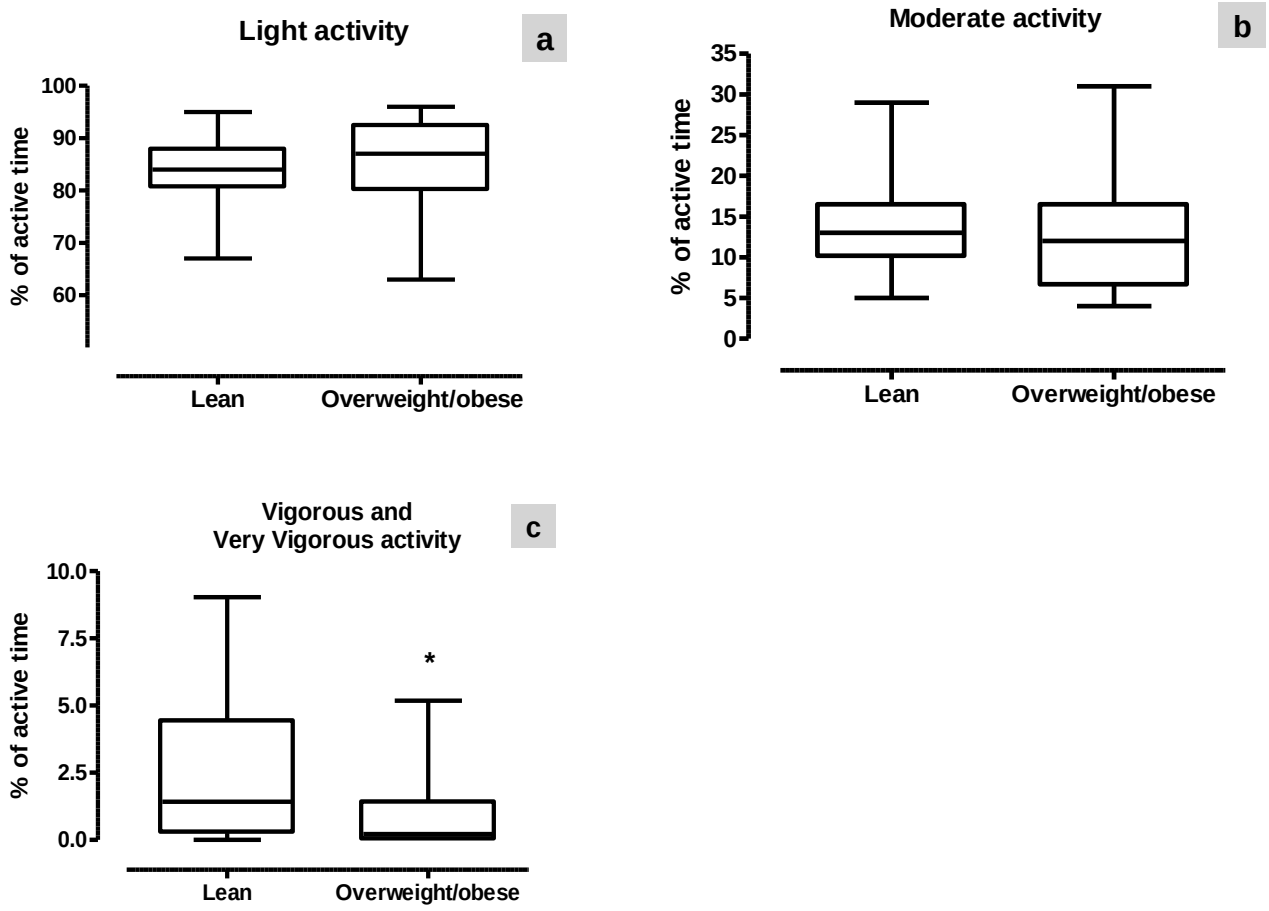
Data in the Figure 3.6 shows that there were no significant differences between lean versus overweight/obese participants in percentage of active time spent in light (lean =84.0±6.4% vs. overweight/obese =85.0±8.7%) and moderate physical activity (lean =13.3±5.7% vs. overweight/obese =13.5±7.9%). However, data of lean participants indicated that they spent significantly higher percentage of active time in vigorous and very vigorous physical activity than overweight/obese group (lean =2.7±2.7% vs. overweight/obese =1.0±1.5%) (Figure 3.6).



*** Indicates that there are significant differences between groups at P< 0.05.**

Figure 3.6 Mean(SD) percentage of registered time spent in different intensity physical activity (lean=33, overweight/obese =21) adults.

The box-and-whiskers plots (Figure 3.7 a, b and c) show the percentage of activity time spent in different physical activity classifications between groups including the range and the middle quartiles as well as the median values of the percentage of the time spent in different physical activity levels.



* Significant differences between groups at *P*<0.05.

Figure 3.7 A box-and-whiskers plot (a, b and c) represents the percentage of activity time spent in different physical activity between lean (n=33) and overweight/obese (n=21) adults.

Table 3-4 showed that there is no significant difference between groups in total registered time. Moreover, there was no significant difference was found between groups in sedentary or active time which is clearly shown in Figure 3.5. The difference between groups in time spent in different physical activity levels was limited. Although, Figure 3.6 indicates that there were no significant differences between groups in most of the physical activity levels, a significant

differences between groups was found in vigorous and very vigorous intensity at $P<0.05$. Regardless of the extreme values shown in Figure 3.7 (c) by some of the participants in each group, lean participants seem to spend more time in vigorous and very vigorous activity than overweight/obese participants.

3.3.3 Habitual physical activity assessed from HR monitoring

Fifty six out of 60 healthy participants completed their HR monitoring in the present study (3 of them (2 males and 1 female) withdrew for personal reasons as already indicated and 1 male had incomplete HR data). Participants were classified into two groups based on their body composition: 34 lean (males n= 16, females n= 18) and 22 overweight/obese (males n= 11, females n= 11). The assessment of physical activity levels was calculated for each participant using the equation of the linear regression between METs and HR obtained from the laboratory treadmill test. Then, the linear regression equation between MET and HR was applied on the measured HR in free-living activity for each participants. Habitual physical activity for each participant was classified based on HR equivalent to sedentary <1.5MET, light $\geq$1.5-3MET, Moderate $\geq$3-6MET, vigorous $\geq$6-9MET and very vigorous >9MET. Table 3.6 shows the measured habitual physical activity using HR monitoring comparing lean group versus overweight/obese group.

Physical activity data of both groups is shown in Table 3.6. The results found that there are significant differences between groups in number of days, registered time, and in each physical activity level except in moderate activity.

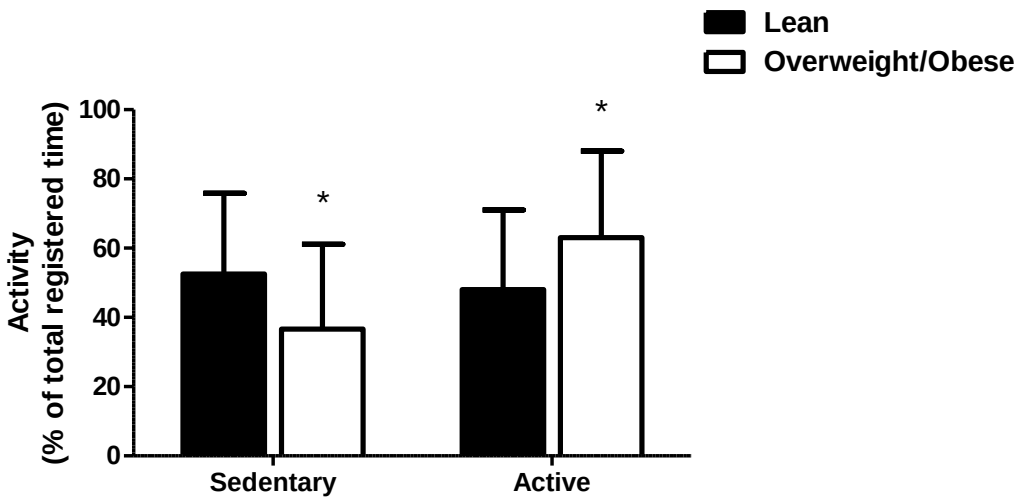
Table 3.6 Physical activity data of 56 participants (mean ±SD) for Lean n= 34 and Overweight/obese n=22.

PA Parameters	**Lean**	**Overweight/obese**
Number of Days	8.4±2.7	6.9±2.2
Registered Time (Minutes)	778.1±85.2	697.0±63.9*
Sedentary (Minutes)	410.6±187.0	254.1±174.8
Light (Minutes)	190.1±90.9	311.1±140.7
Moderate (Minutes)	130.4±94.2	117.1 ±81.8
V & V Vigorous (Minutes)	47.0±.38.6	15.6 ±24.7**

* indicates that there is a significant differences between groups at $P<0.05$.

** indicates that there is a significant differences between groups at $P<0.01$.

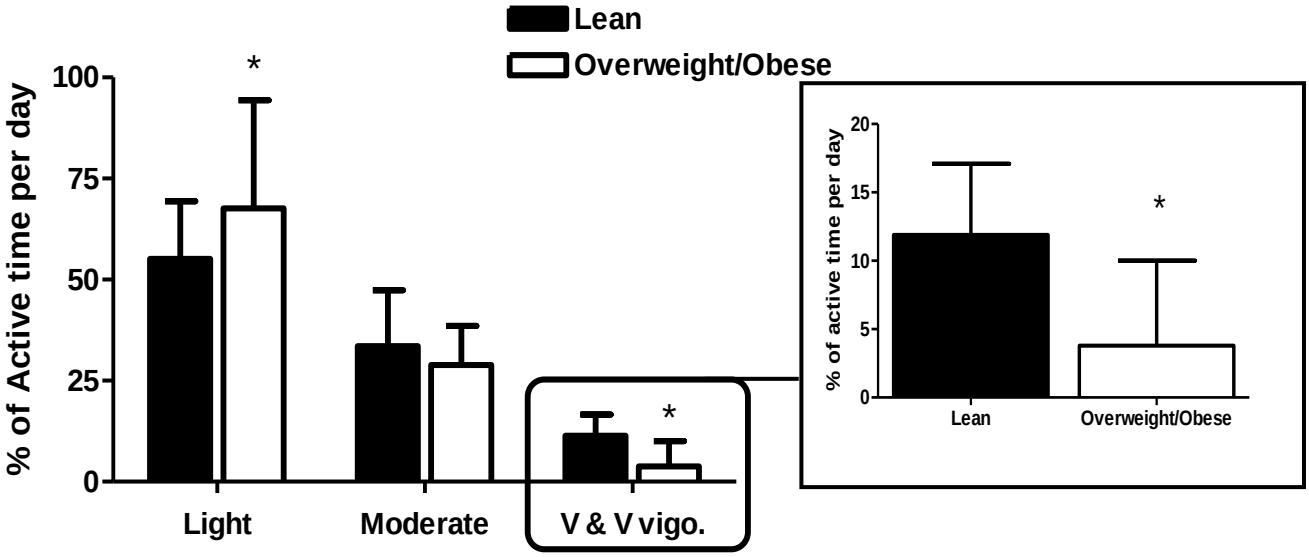
Data indicated that there is a difference between groups in registered time. Therefore, percentages of registered time spent in each physical activity level were examined in order to normalize the data for statistical analysis. Data in Figure 3.8 showed that physical activity data that was assessed by HR showed that there were significant differences between lean and overweight/obese participants in percentage time spent in sedentary and active behaviours per day. Particularly, overweight/obese participants spent less time in sedentary behaviour (overweight/obese =36.6±24.5% vs. lean =52.0±23.4%) and more time in active behaviour (overweight/obese =63.4±18.1% vs. lean =48.0±13.9%) comparing to lean participants.



*** Indicates that there are significant differences between groups at P< 0.05.**

Figure 3.8 Comparing the percentage of sedentary and activity time between lean (n=34) and overweight/obese (n=22) adults.

However, Figure 3.9 clearly showed that most of the time spent by overweight/obese participants in activity time was in light activities (lean =55.1±14.3% vs. overweight/obese =67.6±26.8%). In contrast, lean participants recorded significantly more percentage of active time in vigorous and very vigorous activity than overweight/obese participants (lean =11.4±5.2% vs. overweight/obese =3.8±6.2%). Interestingly, both groups were not significantly different from each other in moderate-intensity physical activity (lean =33.5±13.9% vs. overweight/obese =28.9±9.6%).



* Significant different from Lean at *P*<0.05

Figure 3.9 Comparing between groups in percentage time spent in different physical activity levels from the total activity time for 56 adults lean (n=34) vs. overweight/obese (n=22).

In general, the differences between groups in moderate intensity of physical activity was absent in results of all physical activity assessment methods (AG & HR). On the other hand, the results of all physical activity assessment methods confirm that lean spent significantly more time in vigorous and very vigorous than overweight/obese group. The question is: do participants in both groups meet the recommended physical activity guidelines?

3.3.4 Physical activity status against recommended guidelines

The results of both groups were compared to the recommended physical activity for healthy adults published in 2007 by American College of Sports Medicine (ACSM) and American Heart Association (AHA) (30 minutes on 5

days per week, or vigorous intensity of aerobic physical activity for a minimum of 20 minutes on 3 days per week, or a combination of moderate and vigorous intensity to meet this recommendation) and to the recommended physical activity guidelines for healthy adults by Scottish Health Survey and Health Education Population Survey (SHeS & HEPS) (30 minutes of moderate-intensity or more on five days or more per week). Furthermore, the groups were also compared to the recommended guidelines of physical activity for the purpose of weight loss issued by the US Department of Health and Human Services (DHHS) in 2008 (performing 300 minutes of moderate-intensity, or 150 minutes of vigorous aerobic physical activity or more per week). All results are summarised in Table 3.5.

Table 3.5 Percentage of participants who met ACSM & AHA 2007, SHeS & HEPS 2008 and US DHHS 2008 guidelines. Lean (n=33), overweight/obese (n=22), and all groups (n=55).

Recommendations for health and disease prevention	**Lean**	**Overweight/Obese**	**All groups**
American College of Sports Medicine (ACSM)	60.6%	38.1%	51.9%
Scottish Health Survey (SHeS) and Health Education Population Survey (HEPS)	51.5%	28.6%	42.6%
Recommendations for weight loss	**Lean**	**Overweight/Obese**	**All groups**
US Department of Health and Human Services (DHHS)	39.4%	14.3%	29.6%

3.4 Discussion

3.4.1 Habitual physical activity assessed by Actigraph using 1min bout and 10mins bout methods

In the present study, habitual physical activities of 57 participants (23 lean and 34 overweight/obese) were assessed using an objective instrument (AG). Data of the habitual physical activity obtained from AG was analysed in two different methods (1min bout and 10min bout). Results from both methods showed that there were no significant differences found between lean and overweight/obese participants in the percentage of registered time spent neither in sedentary and active physical activity nor in the percentage of active time spent in light and moderate intensity activities. The only differences between groups was the significantly higher percentage of active time spent in vigorous and very vigorous physical activity by the lean group compared to the overweight/obese group ($P<0.05$). In terms of counts per minute, there were no significant differences between groups in total counts per minute in both methods (1min and 10min bouts). Similar findings have been observed in previous studies. Tyron *et al.* (1987) and Meijer *et al.* (1992) in two different studies compared activity counts between obese and non-obese adults (Tyron, 1987; Meijer *et al.*, 1992). Both studies found that there were no significant differences in activity counts between groups.

The findings of the present study are, partly, different with the findings in some of the previous studies and partly agree with others. For example, Ekelund *et al.* (2002) used both accelerometer and DLW methods and found that obese adults spent significantly less time in moderate physical activity compared to

the lean participants (Ekelund *et al.*, 2002). In another study, an accelerometer and an inclinometer (an instrument that measure angles and elevation) were employed; the results showed that lean participants stood for 2 hours longer per day than obese participants (Levine *et al.*, 2005). Furthermore, other studies have found that differences between obese and non-obese adults are found only on weekends but not on weekdays. In a previous study, Cooper *et al.* (2000) studied 41 lean (BMI<25), 31 overweight (BMI 25-29.9) and 12 obese (BMI $\geq$30) adults using Actigraph. They found that there was no significant differences between lean and overweight in counts per minutes or in any time spent in moderate or vigorous physical activity (Cooper *et al.*, 2000). They, also, concluded that non-obese adults spent significantly more time in physical activity of at least moderate intensity than obese on weekends; however, there were no significant differences found between groups in time spent in moderate activity on weekdays (Cooper *et al.*, 2000). Furthermore, Cooper and colleagues found that obese adults spent significantly less time in vigorous physical activity than non-obese adults on both weekdays and weekends (Cooper *et al.*, 2000). Interestingly, Cooper and colleagues (2000) used Freedson's cut-points for adults (Freedson *et al.*, 1998), which are the same cut-points that were used in the present study.

Actually, there are number of reasons that may lead to these differing results including the results of the present study. Firstly, some of the overweight/obese participants were active and on the other side there were some of the lean participants who were not active enough. For instance, in the present study, over 73% of overweight were performing more than 30 minutes of MVPA per

week. In contrast, over 20% of lean were performing less than 30 minutes of MVPA per week. Another reason could be the nature of these studies (assessing physical activity) as the benefits that may be gained from taking part in these studies include some health information such as assessing how fit people are, or the estimated calories expended per week … etc. might attract some of the active overweight participants who are already engaged in an exercise programme. As mentioned earlier, the overweight/obese participants may have tried to show that they were physically active when their activities were monitored (Lightman *et al.*, 1992; Jakicic *et al.*, 1998).

Although the 1min bout method may be a beneficial for adults to increase physical activity levels and decrease body mass index (BMI) and waist circumference, performing recommended 10min bouts has been reported to provide more advantage over the 1min bout method (Strath *et al.*, 2008). In a recent study, Strath and colleagues (2008) concluded that 10mins bouts of MVPA and 1min bouts of MVPA are independently associated with BMI and waist circumference in adults, after controlling for confounding variables. However, the association between BMI reduction and the 10mins bout method was about four times greater than the 1minute bout method, and about three times greater than the 1min bout in waist circumference reduction (Strath *et al.*, 2008). The finding in the present study showed that both methods (1min bout and 10mins bout) provided similar results between groups in terms of percentage of active time spent in moderate, MVPA and vigorous or more intensity.

If both groups are doing these levels and amounts of physical activity during their lifestyle, then some of them (only between 52% and 43%) were doing enough physical activity for health promotion. However, overweight/obese participants may need to increase their physical activity to meet the recommended guidelines in order to reduce their weight (body fat). The Department of Health and Human Services (DHHS) recommended at least 300 minutes (5 hours) of moderate-intensity, or 150 minutes (2.5 hours) of vigorous-intensity per week, or an equivalent combination of moderate-and vigorous-intensity aerobic physical activity for the aim of additional health benefits and for weight loss (U.S. Department of Health and Human Services, 2008). Recently, the American College of Sports and Medicine (ACSM) updated its Position Stand on weight loss and prevention of weight gain for adults (18-65yr). In 2009, the ACSM concluded that scientific evidence supports 150- 250 minutes per week of moderate-intensity aerobic physical activity to be effective to prevent weight gain, and may provide modest weight loss. However, a greater amount of moderate-intensity physical activity (250 mins.wk^{-1} or more) is recommended for long-term weight loss and to prevent weight regain (Donnelly *et al.*, 2009). The Institute of Medicine (IOM) reported recommendations about time spent in moderate intensity for weight loss and prevention of weight regain (Institute of Medicine of the National Academies. 2002). The IOM recommended at least 60 minutes of moderate intensity of physical activity per day for prevention of weight gain and to increase additional weight-independent health benefits (Institute of Medicine of the National Academies. 2002). In the present study both groups did not meet the IOM recommendations even in the lean group when physical activity levels were

assessed by AG (in both methods 1min & 10mins bouts). However, the lean group was closer to these recommendations than the overweight/obese group in both methods. When physical activity was assessed by HR method, both groups met (more than 2 fold) the recommended time in MVPA.

Based on the recent scientific investigations and recommendations, all participants - in both groups - in the present study met the recommended guidelines for improve and maintain health ($\geq$ 30 min of Moderate-intensity of physical activity in $\geq$5 days) (Table 3.5). However, for the purpose of weight loss and for prevention of weight regain recommendations ($\geq$60 mins.d^{-1} of MVPA in $\geq$5 days), there were only 39.4% of lean and 14.3% of overweight/obese met the recommended guidelines in the current study (see Table 3.5). Thus, overweight/obese participants should increase their physical activity to meet the recommended guidelines in order to reduce body weight (body fat) and the risk of chronic diseases such as cardio vascular diseases and diabetes (Panagiotakos *et al.*, 2003; Fang *et al.*, 2003). In previous studies, researchers have found that coronary heart disease is inversely associated with high level of physical activity (vigorous-intensity) for middle-aged men (Epstein *et al.*, 1976; Morris *et al.*, 1953; Morris *et al.*, 1990). For example, Epstein and colleagues (1976), investigated the relationship between vigorous exercise, other recognized coronary risk factors, and electrocardiographic evidence of myocardial ischaemia within a group of middle age males (509 men) of similar occupational and socioeconomic status. They found that middle-aged men whom reported vigorous exercise in their leisure time (125 (25%)) had significantly fewer electrocardiographic abnormalities than the men who did not

report vigorous exercise (P<0.02). In the present study, 45.5% of lean and only 14.3% of overweight/obese met an average of ≥20minutes of vigorous-intensity or more for at least 3days per week or a combination of 2days of ≥20minutes of vigorous activity and ≥20minutes of moderate-intensity on 2 more days. Table 3.4 and Figure 3.6 indicated that the lean group performed vigorous and very vigorous activity (Vigo & Vvigor.) significantly more than the overweight/obese group (more than twice the time). Furthermore, the time spent by lean in Vigo & Vvigor was about 9.6 mins.d^{-1} and by overweight/obese was 3.6 mins.d^{-1} which means that the lean met the recommended guidelines in terms of Vigo & Vvigor physical activity (about 67mins per week) whereas overweight/obese participants did not (only about 23mins per week).

Although overweight/obese participants showed they were active and met the guidelines for health promotion, they may be at risk of some chronic diseases due to lack of vigorous physical activity. Tremblay *et al.* (1994) concluded that vigorous intensity exercise found to be associated with lipid balance to a greater extent than exercise of low to moderate exercise intensity. Moreover, the metabolic adaptations that occur in the skeletal muscle in response to the high intensity training programme appear to favour the process of fat oxidation (Tremblay *et al.*, 1994). The contribution of the present study is to assess physical activity levels using objective measures as a step to understanding the association between physical activity status and adiposity. Thus, more studies to investigate the relationship between physical activity -especially the importance of vigorous activity- and some of the cardiovascular and metabolic diseases risk markers are suggested.

3.4.2 Habitual Physical Activity by HR Monitor

The habitual physical activity of the 56 participants was assessed by HR monitoring as an objective physiological based measurement. The results showed significant differences between groups in sedentary and active time (Figure 3.8). Comparing to the results obtained from AG, the differences come from the overweight/obese group as they showed a lower time spent in sedentary activity and more time in light activity than the lean (see Figure 3.9). However, the lean group showed similar percentage of registered time spent in sedentary and active time from both measurement methods (HR or AG (10mins bout)). It is important to note that the disagreement between AG (1min and 10mins bouts) and the HR method was mainly due to the fact that the HR method was developed based on a limited range of physical activity intensities (only the lab exercise test (walking at 3, 4.5 & 6 $km.h^{-1}$) which, in turn, provided higher values of time spent in each physical activity level especially in overweight/obese participants. Furthermore, the higher activity values with the HR method were probably due to some other physiological or psychological factors such as emotion, diet status, and/or relatively high body weight which may affect the heart rate in overweight/obese participants.

The HR method and AG method (1min bout and 10mins bout) provided some different results. However, similar results were found in AG (in both methods) and HR method in moderate-intensity level as well as in vigorous and very vigorous-intensity between groups. Specifically, Figure 3.6 and Figure 3.9 clearly show that both measurements (HR and AG (10mins bout)) showed that there were no significant differences between groups in moderate-intensity

whereas the significant differences were found between groups in vigorous and very vigorous-intensity.

In fact, both methods have limitations but the AG method is preferred for assessing habitual physical activity more than the HR method because AG has been found to be valid and reliable in assessing physical activity level in adults and provides consistent output (Freedson et al. 1998). Thus, AG is recommended over HR method for further studies.

Although, the present study was well controlled in terms of procedures and design, the results of the present study would be more conclusive if the sample size was equal in each group, or if a larger number of participants was included in each group. Due to the time limit given to complete the current study, the sample size of the current study was below the number required to generalise the results, as the power calculation for the sample size indicated that the required number of sample is 37 participants (at power of 80%) or 56 participants (at power of 90%) for each group (lean vs. overweight/obese).

3.5. Conclusion

In the present study, AG and HR were used as objective instruments to assess habitual physical activity for adults including lean and overweight/obese participants.

The results indicated that all groups met the recommended guidelines for health and disease reduction. However, overweight/obese participants as a group did not meet the recommended period of time spent per week for the

purpose of losing weight. Moreover, the results showed that there are no significant differences between groups in terms of time spent in moderate-intensity activity. On the other hand, data obtained from AG using 10mins bout method as well as the data obtained from HR showed that there were significant differences between groups in proportion of time spent doing vigorous and very vigorous-intensity activity. The lean group met the recommended vigorous and very vigorous-intensity guidelines whereas the overweight/obese did not and this may increase their chance of being at risk of some chronic diseases and may limit their chance of losing fat mass. Although the number of the participants was below the required number to compare lean versus overweight/obese groups, the results of the well controlled study raised some issues that appear to be important for tackling obesity. Thus, overweight/obese participants are recommended to perform more time in moderate to vigorous physical activity in order to lose weight (body fat) as well as reduce the chance of being at risk of some chronic diseases that are caused by obesity. A

Chapter 4. Association between physical activity status and cardiovascular and metabolic disease risk markers in adults.

4.1 Introduction:

Over the last two decades the prevalence of overweight/obesity has increased and found to be one of the health problems (Visscher *et al.*, 2002), and this is partly related to lower levels of physical activity (Jebb and Moore, 1999). Obesity and being physically inactive may cause a number of health problems such as cardiovascular disease (CVD) (Epstein *et al.*, 1976; Morris *et al.*, 1953), artery disease (Morris *et al.*, 1990) and metabolic disease (Panagiotakos *et al.*, 2003; Fang *et al.*, 2003) as well as increase incidence of some cancer (Brown *et al.*, 2007). On the other hand, being physically active can reduce the risk of some of the metabolic diseases (Lee *et al.*, 1999) and some cancers (Farrel *et al.*, 2007) as well as reduce the risk morbidity and mortality even among obese adults (Blair and Brodney, 1999).

The negative changes in the concentration of some of the traditional blood markers such as total cholesterol, high density of lipoprotein (HDL), low density of lipoprotein (LDL), very low density of lipoprotein (VLDL), triglycerides and some types of fatty acid such as omega 3 (n-3) and omega 6 (n-6) have been found as cardiovascular diseases risk markers and fasting insulin and glucose as metabolic disease risk markers (Jakicic *et al.*, 1993; Cho *et al.*, 2009; Thorseng *et al.*, 2009). Additionally, newer blood markers such as adipokines including leptin (Jurimae *et al.*, 2003), adiponectin, interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP) (Engeli *et al.*, 2003) and tumor necrosis

factor-alpha (TNF-α) have been investigated to explore the association between adiposity and some of the chronic disease (Engeli *et al.*, 2003; Monzillo *et al.*, 2003). The association between adiposity and physical activity and total cholesterol, HDL, LDL, VLDL, and triglyceride -as traditional factors- has been well studied (Jakicic *et al.*, 1993; Brown *et al.*, 2007; Laaksonen *et al.*, 2002). However, the influence of these traditional blood lipids as cardiovascular disease markers are partly depending on other factors such as omega 3 (n-3) and omega 6 (n-6) levels (Lands, 2003). In a recent study, Thorseng and colleagues (2009) found that n-3 fatty acids may have a protective effect against insulin resistance. They, also, concluded that the role of the other factors such as physical activity, diet, energy intake and socio-economic status deserves more investigations (Thorseng *et al.*, 2009).

On the other hand, the association between the body composition and habitual physical activity and some of the newer risk markers such as hsCRP, IL-6, TNF-α, leptin and adiponectin need to be investigated (Engeli *et al.*, 2003; Monzillo *et al.*, 2003). Most of the research that has studied the relationship between some of the cardiovascular and metabolic risk markers and physical activity has used subjective methods such as questionnaire (Cho *et al.*, 2009) or interview rather than objective measurement methods such as accelerometry (Monzillo *et al.*, 2003) (see Chapter 1 for detail review of these risk markers). To date, there are few studies that investigated the association between the traditional cardiovascular risk markers such as total cholesterol, HDL, LDL, VLDL, triglyceride and fatty acids (n-3 and n-6) or metabolic risk markers such as insulin, fasting blood glucose and HOMA-IR and body composition and

physical activity status in adults using objective measurement methods. In addition, very few studies have investigated the health benefits of performing low physical activity below the recommended guidelines –but not sedentary- in terms of making positive changes in some of the traditional and new cardiovascular and metabolic risk factors (Brown *et al.*, 2007). Furthermore, there is no study that has examined the impact of high and low physical activity status using objective measurements such as accelerometry method on the newer cardiovascular and metabolic markers such as adiponectin, leptin, IL-6, hsCRP, TNF-alpha, and more novel mechanistic cardiovascular disease risk markers such as omega-3 and omega-6.

4.1.1 Aims of the study

1. To investigate the association of key metabolic and cardiovascular risk markers with body composition and physical activity status in apparently healthy adults.
2. To investigate whether or not there is health benefits of physical activity below and above the recommended physical activity guidelines for adults regardless of adiposity status.

4.2 Materials and Methodology:

Based on the power calculation, the required number of participants was 30 subjects (at power of 80%) in each group. However, only fifty five participants (lean n=33 (male=15, female=18) and overweight/obese n=22 (male=12,

female= 10)) were able to provide blood sample for the current study. Participants were classified into one of the groups (lean or overweight/obese) based on the classification method that was provided by ACSM's guidelines (American College of Sports Medicine, 2005) (see chapter 3, section 3.2.21). The fifty five participants were asked to attend early morning (7- 9am) after an overnight fast to the laboratory on one occasion. A total blood sample of 10ml was collected from a peripheral vein. The habitual physical activities and physical measurements of all participants were assessed by objective instruments as described in Chapter 3. All blood samples were collected into vacutainer tubes containing K_3EDTA by a trained person from an antecubital vein with the person in a seated position. The blood sample was centrifuged at 4^{o}C for 10 minutes to isolate plasma. The plasma was stored in each of 6 aliquots for subsequent analyses:

- 1ml for fasting triglycerides, total cholesterol and free fatty acid (FFA).
- 0.5ml for fasting glucose.
- 1ml for leptin and adiponectin.
- 1ml for tumor necrosis factor-alpha (TNF-α), high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6).
- 0.5ml for insulin.
- 1ml for omega-3 and omega-6.

ELISA Assay Procedure:

The Enzyme-Linked Immunosorbant Assay (ELISA) assay procedures were applied to leptin, adiponectin, TNF-α, hsCRP, IL-6, and insulin in the present

study. The Enzymatic Assay procedures were applied to triglyceride, total cholesterol, free fatty acid and glucose. Lipid profile ratio of arachidonic acid to eicosapentaenoic acid (ARA/EPA), [also known as (20:4n-6)/(20:5n3)] and the ratio of % n-3 to the total highly unsaturated fatty acid (HUFA) were assayed using the Ideal Omega Test provided on online (Ideal Omega test (no publication date). Available: http://www.idealomegatest.com). The procedure of insulin ELISA will be described in detail as an example for the leptin, adiponectin, TNF-α, hsCRP, IL-6, and insulin. Glucose oxidase assay procedure will be described as an example of the enzymatic assay of triglyceride, total cholesterol, free fatty acid and glucose. Omega 3 (GC-MS) assay will be described as an example of Omega 3 and Omega6.

ELISA Insulin assay:

Sufficient rows on the plate were selected to accommodate standards, controls and all test samples. Then all rows were fitted into the holding frame. Afterword: each standard, control and participant sample (50µl) was pipetted into the appropriate wells. An amount of 50µl of Anti-Insulin HRP was dispensed and conjugated into all wells then incubated for 30 minutes at room temperature on a horizontal shaker set at 700 ±100 rpm. Then each well was washed three times with 0.4 ml of washing solution. The washing solution was removed carefully. The plate was inverted on clean blotting and tapped firmly paper to remove any remaining liquid. Following the washing step, an amount of 200 µl of freshly prepared substrate solution was applied into each well and incubated them for 15 minutes at room temperature on a horizontal shaker set at 700 ±100 rpm, avoiding direct sunlight. The substrate reaction was stopped by

adding 50 µl of stopping reagent (H2SO4 1.8N) into all wells and briefly contents were mixed by gently shaking the plate. Within 60 minutes after stopping, the optical density was read at 450 and 490 nm (reference wave length 600-650 nm) using a microtiterplate reader.

Calculation of Results

After reading the microtiter plate at 450 nm (reference filter: 650 nm), a standard curve was constructed using all standard points for which absorbencies are < 1.5 OD unit.

The OD was plotted on the ordinate against the standard concentrations on the abscissa using MS excel programme and the curve was drawn by connecting the plotted points with straight lines. Insulin concentrations of samples or controls were determined for which absorbance was not greater than those of the last standard plotted at 450 nm.

If any control or sample has an absorbance greater than the absorbance of the last standard read at 450 nm, a second reading at 490 nm (reference filter: 650 nm) was taken. The segment of the curve drawn between the last standard read at 450 nm and the most concentrated standard was considered at 490 nm. The concentration of samples and controls for which absorbance included in this segment were read at 490 nm. The same equipment was used for both readings at 450 nm and 490 nm. The readings at 490 nm are only for off-scale values at 450 nm (above 1.5 OD unit.) and the readings at 450 nm were not replaced for values below 1.5 OD unit. If any sample reading was greater than the highest standard, then it was diluted appropriately with zero standards

(Standard 0) and re-assayed and the result would be multiplied by the corresponding dilution factor.

Glucose Assay (using glucose oxidase enzyme)

Glucose ($C_6H_{12}O_6$) is a fuel molecule in biology. Glucose oxidase enzyme was used as a sample of the enzymatic assay (Boston *et al.*, 2003; Hong *et al.*, 1996). Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red - violet quinoneimine dye as indicator. The reaction principle is:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone+phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

1. Procedure for glucose god-pap assay without deproteinisation

Wavelength	500 nm, Hg 546 nm
Cuvette	1 cm path length
Temperature	15-25°C
Measurement	against reagent blank

Pipette into test tubes

	Macro		Semi Micro	
	Standard or sample	Reagent Blank	Standard or Sample	Reagent Blank
Standard or sample	20µl	---	10 µl	---
Reagent	2000 µl	2000 µl	1000 µl	1000 µl

Then they were mixed and incubated for 25 min at 15-25°C. The absorbance of the standard (A standard) and the sample (A sample) were measured against the reagent blank within 60 minutes. The time interval from sample addition to read time was the same for Standard/Control and Sample.

Calculation

$$\text{Glucose concentration (mmol/L)} = \frac{A_{sample}}{A_{standard}} \times 5.55$$

Linearity

The test was considered as linear up to a glucose concentration of 22.2 mmol/L. If samples above this concentration then they were diluted 1+2 with distilled water and the result was multiplied by 3.

Normal values

The normal range of the fasting glucose is between 4.2 – 6.4 mmol/L(Sung *et al.*, 2007).

4.2.1 Data analysis and Statistics

Data was sorted and stored in different excel worksheets. All measured variables were used to compare lean versus overweight/obese and active versus low active adults.

Statistical analyses were conducted using SPSS software version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). An alpha level of 0.05 was used for all analyses to indicate statistical significance. Data are summarised as mean differences with confident interval (95% CI) and as means and standard deviation (±SD). Independent-samples t-tests were used to compare the measured values for lean versus overweight/obese groups as well as between active versus low active groups. Pearson correlation coefficients were used to assess the strength of relationship between variables.

4.3 Results

4.3.1 Association between cardiovascular and metabolic risk factors and body composition in adults.

Fifty five out of the fifty seven participants who completed their participation in measuring free-living physical activity were able to provide 10ml of blood sample. The 55 participants were grouped into two groups based on body composition: 33 lean (male n= 15, female n= 18) and 22 overweight/obese (male n= 12, female n= 10). Physical characteristics of the participants for each group are summarised in table 4.3.1. Data is represented as mean(±SD). The differences between groups were significant in body mass (BM), BMI, body fat percentage (BF%) (all $P<0.01$), fat free mass (FFM) ($P=0.43$), height ($P<0.05$), and mean arterial pressure (MAP) ($P<0.01$).

Table 4.3.1 Physical characteristics expressed as means ±SD for 55 adults, (Lean n=33, Overweight/obese n= 22)

Physical characteristics	Lean	Overweight/Obese
Age (yrs)	36.2±10.4	40.0±8.4
Body Mass (Kg)	66.8 ±8.9	91.0 ±13.2 **
Height (cm)	168.8 ±9.6	174.1 ±8.5 *
BMI ($Kg.m^{-2}$)	23.3 ±1.9	30.1 ±4.0 **
Body Fat%	22.1 ±7.3	35.6 ±12.4 **
Fat Free Mass (Kg)	51.7 ±9.4	57.8 ±12.4 *
Mean arterial pressure (MAP) (mmHg)	87.0±8.8	95.3±7.4 **
Resting heart rate (bpm)	65.8 ±9.2	70.7 ±10.7
Predicted VO_{2Max} ($L.min^{-1}$)	3.2 ±0.8	3.0 ±0.7
Predicted VO_{2Max} ($ml.kg^{-1}.min^{-1}$)	48.1 ±10.5	32.8±6.3 **
MVPA (min/w)	378.0±141.4	332.5±212.1

* indicated that there is a significant differences between groups at P< 0.05.

** indicated that there is a significant differences between groups at P< 0.01.

Actual number of days and registered time (mins/d) are reported in Table 4.3.2 as mean (±SD). The registered time between groups was significantly different (*P*=0.03). Thus, all other physical activity status data was reported as percentage of time spent in each physical activity level in order to normalise the compared values. Data in Table 4.3.2 shows no differences between groups in percentage of registered time spent in sedentary (<light physical activity) and active behaviour (≥light physical activity). Moreover, there were no significant difference found between groups in percentage of activity time spent in each of

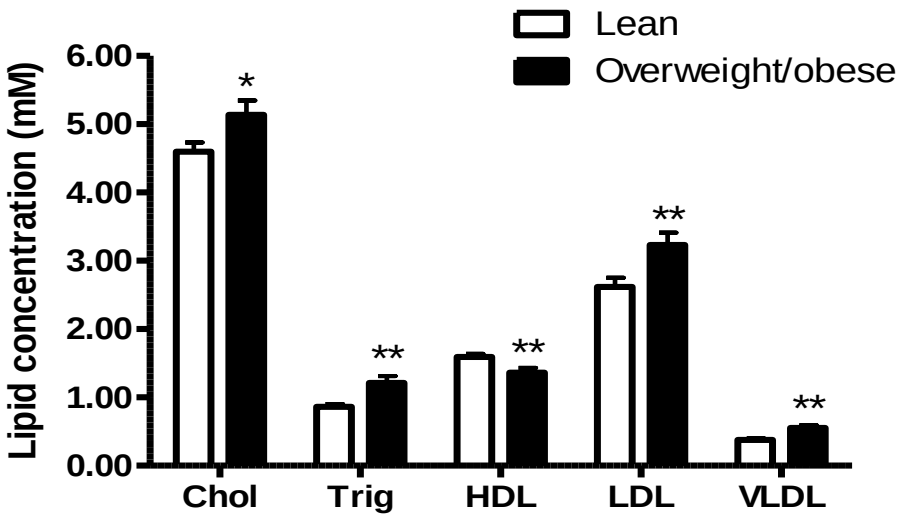
the physical activity levels (all *P*>0.05) although the data were tending to near significance for vigorous activity (P=0.09) and combined vigorous and very vigorous activity (P=0.06).

Table 4.3.2 Mean (±SD) of number of days and registered time (mins/d) and percentage of time spent per day in each of the physical activity levels. n=55 adults (Lean n=33 and Overweight/obese n=22).

PA Parameters	Lean	Overweight/obese	P value
Number of Days	9.0 ±2.9	7.6 ±2.7	0.08
Registered Time (Mins/d)	772.3 ±61.9	737.2 ±49.0 *	0.03
Sedentary (% of registered time)	54.5 ±6.6	54.9 ±10.2	0.86
Activity (% of registered time)	45.5 ±6.6	45.1 ±10.2	0.86
Light (% of active time)	84.1 ±6.5	85.6 ±8.0	0.43
Moderate (% of active time)	12.9 ±5.9	13.0 ±6.8	0.94
Vigorous (% of active time)	2.5 ±2.7	1.3 ±1.8	0.09
Very Vigorous (% of active time)	0.3 ±0.7	0.1 ±0.1	0.22
Moderate to Vigorous (MVPA) (%)	15.7 ±6.5	14.3 ±7.9	0.49
Vigorous to Very Vigorous (V&VvPA) (%)	2.7 ±2.9	1.4 ±1.9	0.06

* Differences are significant between groups at P< 0.05.

As it is clearly illustrated in Figure 4.3.1, the overweight/obese group has a higher concentration of total cholesterol, triglyceride, LDL and VLDL than the lean group. In contrast, overweight/obese group has lower level of HDL than lean group. The differences between groups were significant at *P*<0.01 except in total cholesterol as the difference was significant at *P*<0.05.

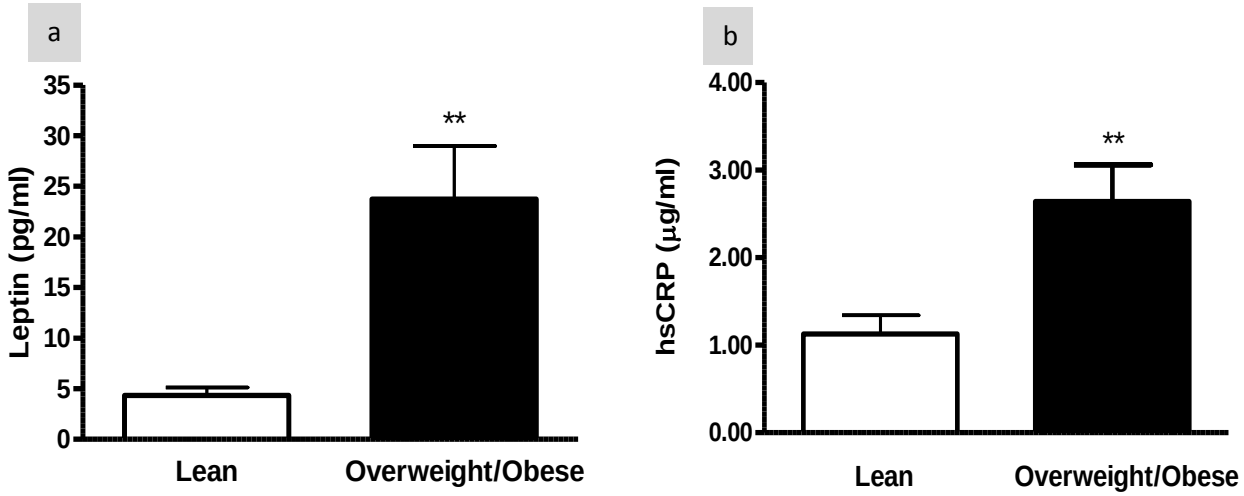


* Differences are significant between groups at P<0.05.

** Differences are significant between groups at P<0.01.

Figure 4.3.1 Mean (SD) of blood lipid concentrations (total cholesterol, triglyceride, HDL, LDL and VLDL) for 55 adults (Lean n=33, Overweigh/obese n=22).

Furthermore, some of the cytokine markers (leptin and hsCRP) were significantly different in overweight/obese group versus lean group (P<0.01) where others (adiponectin, IL-6 and TNF-α) were not (P=0.92, 0.95, 0.37 respectively) (see Table 4.3.3 for Mean±SD). Figure 4.3.2 shows the differences between groups in leptin (a) and hsCRP (b).

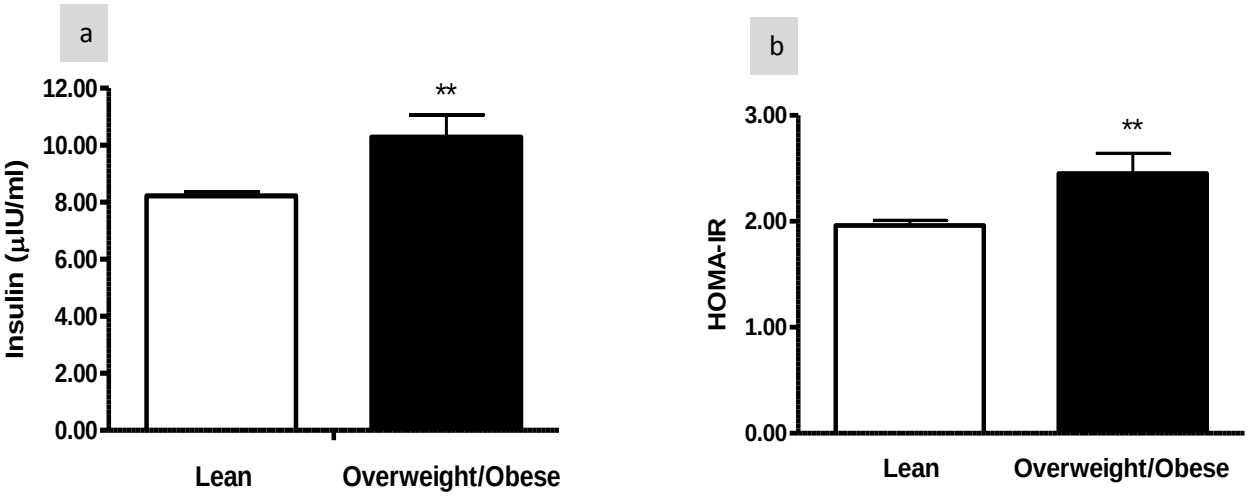


* Differences are significant between groups at P< 0.05.

** Differences are significant between groups at P< 0.01.

Figure 4.3.2 Mean(±SD) of leptin (a), and hsCRP (b) for 55 adults (Lean n=33, Overweigh/obese n=22).

Similarly, Figure 4.3.3 illustrates that the results showed significant differences between groups in insulin and HOMA-IR (homeostatic model assessment of insulin resistance) (P<0.01), but not in fasting glucose (P=0.85). Overweight/obese participants have higher fasting insulin and HOMA-IR than lean participants.

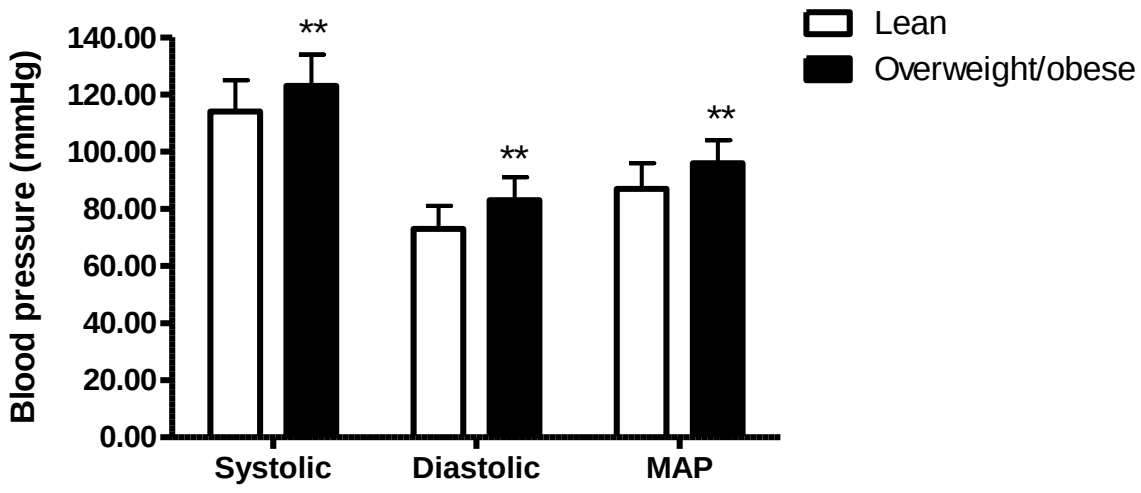


* Differences are significant between groups at P< 0.05.

** Differences are significant between groups at P< 0.01.

Figure 4.3.3 Mean(SD) of insulin (a), and HOMA-IR (b) for 55 adults (Lean n=33, Overweigh/obese n=22).

There were significant differences between groups in all blood pressure results (*P*<0.01). However, Figure 4.3.4 illustrates that the mean values of all blood pressure results were within normal levels in both groups.



* Differences are significant between groups at P< 0.05.

** Differences are significant between groups at P< 0.01.

Figure 4.3.4 Mean(SD) of blood pressure (systolic, diastolic, MAP) for 55 adults (Lean n=33, Overweigh/obese n=22).

The results of the blood analyses are summarised in Table 4.3.3. The comparison between groups is represented as mean (±SD) and as mean differences with 95% confidence interval (95% CI). The results showed that there are significant differences between groups (lean vs. overweight/obese) in most of the cardiovascular and metabolic disease risk markers. In contrast, there were no significant differences found between groups in adiponectin, IL-6, TNF-α, fasting glucose, n-6:n-3 ratio (ARA:EPA) and % n-3 HUFA/Total HUFA.

Table 4.3.3 Mean (±SD) and mean differences (95% CI) of the blood markers for lean and overweight/obese adults (Lean n=33, Overweigh/obese n=22)

Blood markers	Lean	Overweight/Obese	Mean differences (95% CI)
Total Cholesterol (mM)	4.60 ±0.13	5.14 ±0.21	-0.54 (-1.05, -0.32) *
High Density Lipoprotein (HDL) (mM)	1.59 ±0.05	1.36 ±0.07	0.23 (0.05, 0.42) **
Very Low Density Lipoproteins (VLDL) (mM)	0.38 ±0.02	0.55 ±0.04	-0.16 (-0.26, -0.06) **
Low Density Lipoprotein (LDL) (mM)	2.62 ±0.13	3.23 ±0.18	-0.62 (-1.07, -0.16)**
Triglycerides (mM)	0.86 ±0.04	1.21 ±0.10	-0.35 (-0.56, -0.13) **
Adiponectin (pg/ml)	6.37 ±0.64	6.51 ±1.00	-0.11 (-2.48, 2.27)
Leptin (pg/ml)	4.33 ±0.80	23.73 ±5.25	-22.15 (-34.79, -9.51) **
Interleukin-6 (IL-6) (pg/ml)	7.33 ±1.03	7.34 ±2.35	1.55 (-5.00, 5.31)
Tumor Necrosis Factor-alpha (TNF-α) (pg/ml)	5.11 ±5.88	4.43 ±6.13	0.79 (-0.92, 2.51)
hsC-reactive Protein (hsCRP) (µg/ml)	1.13 ±0.21	2.64 ±0.42	-1.47 (-2.42, -0.52) **
Insulin (µg/ml)	8.23 ±0.14	10.29 ±0.78	-2.15 (-3.77, -0.53) **
Glucose (mM)	5.37 ±0.13	5.37 ±0.14	0.038 (-0.35, 0.42)
HOMA-IR	1.96 ±0.05	2.45 ±0.19	-0.49 (-0.88, -0.09) **
n-6/n-3 (ARA/EPA)	7.71 ±3.81	8.34 ±3.93	-0.63 (-2.85, 1.60)
% n-3 HUFA/Total HUFA	31.99 ±9.20	28.53 ±7.49	3.46 (-1.45, 8.37)

* Differences are significant between groups at P< 0.05.

** Differences are significant between groups at P< 0.01.

4.3.2 Association between cardiovascular and metabolic risk factors and physical activity status in adults.

A total of forty seven participants who completed their participation in measuring free-living physical activity were grouped into two groups based on their physical activity levels (High active $\geq$ 300min/w of MVPA (SHeS), Low active <150min/w of MVPA (ACSM)):

22 high active (Lean n= 17, Overweight/obese n= 5)

25 low active (Lean n= 12, Overweight/obese n= 13).

Physical characteristics of the participants are summarised in Table 4.3.3.

The only difference between groups in physical characteristics were found in body mass index (BMI) and body fat percentage (BF%) as well as in the mean arterial pressure (MAP).

Table 4.3.3 Physical characteristics presented as means ±SD for 47 adults (high active n= 22, low active n= 25)

Physical characteristics	High active	Low active
Age (yrs)	33.6±10.8	39.3±8.8
Body Mass (Kg)	71.3±17.6	79.0±14.5
Height (cm)	170.8 ±10.9	170.9 ±9.1
BMI ($Kg.m^{-2}$)	24.2 ±3.4	26.9±4.8 *
Body Fat % (BF%)	22.4 ±7.4	31.1 ±12.8 **
Fat Free Mass (Kg)	54.6 ±12.5	53.1 ±10.2
Mean arterial pressure (MAP) (mmHg)	87.0±7.1	92.5±10.3 *
Resting heart rate (bpm)	67.2 ±9.8	71.2 ±8.7
Predicted VO_{2Max} ($L.min^{-1}$)	3.30 ±0.86	2.83 ±0.43 *
Predicted VO_{2Max} ($ml.kg^{-1}.min^{-1}$)	47.5 ±12.3	36.8±7.8 **
MVPA (min/w)	434.0±119.7	141.1±56 **

* Differences are significant between groups at P< 0.05.

** Differences are significant between groups at P< 0.01.

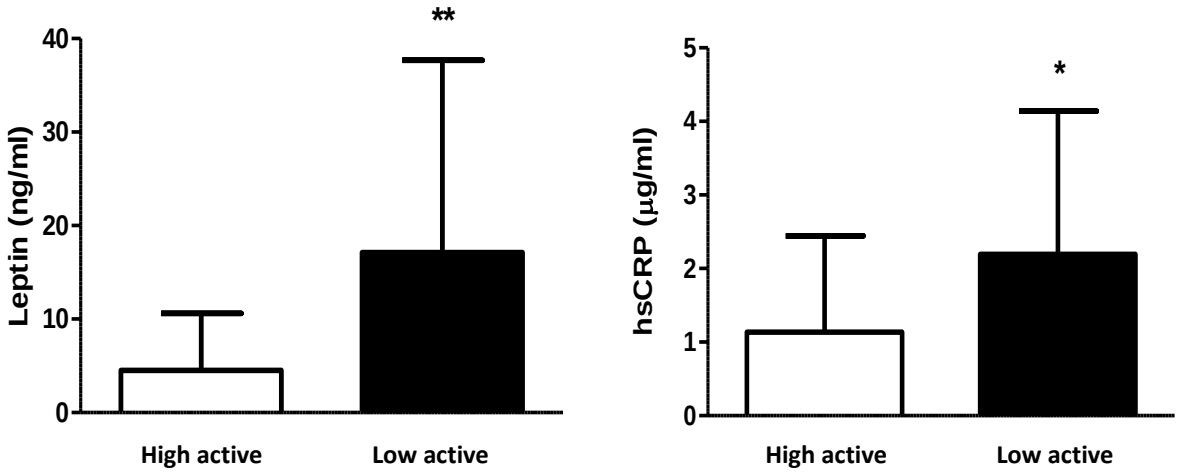
Physical activity status of high active and low active groups is summarised in Table 4.3.4. Number of days and registered time ($mins.d^{-1}$) are presented in Table 4.3.4 as mean (±SD). Physical activity status of both groups is summarised as percentage of time spent in each physical activity level in order to normalise the compared values. Data in Table 4.3.4 shows no differences between groups in percentage of registered time spent in sedentary (<light physical activity) and active behaviour (≥light physical activity). However, there were significant differences between groups in percentage of activity time spent

in each physical activity level, with lower percentage time spent in light activity but higher percentage of time at higher intensities in the high active group.

Table 4.3.4 Mean (±SD) number of days and registered time (mins.d^{-1}) and percentage of time spent per day in each of the physical activity level. n=47 adults (High active n=22 and Low active n=25).

PA Parameters	High active	Low active	P value
Number of Days	9.3 ±2.6	7.4 ±2.8	0.07
Registered Time (min/d)	780.2 ±68.3	749.4 ±47.7	0.08
Sedentary (% of registered time)	56.4 ±7.3	52.8 ±8.6	0.13
Activity (% of registered time)	43.6 ±7.3	47.2 ±8.6	0.13
Light (% of active time)	81.1 ±4.7%	94.2 ±2.1**	0.00
Moderate (% of active time)	15.0 ±5.5%	4.9 ±1.7**	0.00
Vigorous (% of active time)	3.0 ±2.8%	0.9±1.6**	0.00
Very Vigorous (% of active time)	0.40 ±0.90%	0.02 ±0.04*	0.03
Moderate to Vigorous (MVPA) (%of active)	18.5 ±4.9%	5.7 ±2.1**	0.00
Vigo. & V Vigo. (V&VVPA) (% of active)	3.4 ±3.0%	0.9 ±2.9**	0.00

The only differences found between high active versus low active participants for the blood markers were in leptin and hsCRP as well as differences in MAP and diastolic blood pressure. Figure 4.3.5 showed the mean (±SD) values of leptin and hsCRP for each group. The figure clearly shows that low active group has higher level of leptin and hsCRP than high active group (P<0.01, P<0.05 respectively).

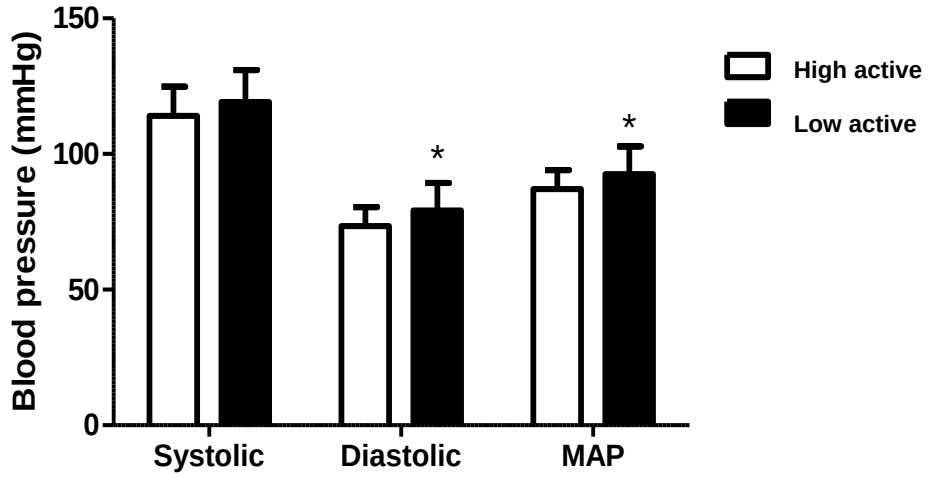


* Differences are significant between groups at P< 0.05.

** Differences are significant between groups at P< 0.01.

Figure 4.3.5 Mean (±SD) of leptin and high sensitivity C-reactive protein (hsCRP) for 47 adults (high active n=22 vs. low active n=25).

On the other hand, Figure 4.3.6 illustrated the mean (±SD) values of the blood pressure measurements (systolic, diastolic and mean arterial pressure (MAP)) for both groups. Although low active group has significant higher MAP and diastolic blood pressure than high active group (P<0.05), all blood pressure measurements for both groups were within normal levels.



*** indicate that there is significant differences between groups at *P*<0.05.**

Figure 4.3.6 Mean (±SD) of blood pressure (systolic, diastolic, MAP) for 47 adults (high active n=22 vs. low active n=25).

Based on the physical activity readiness questionnaire, there was only one low active lean female who was 48yrs and she indicated that she had high total cholesterol. Therefore, her blood sample was excluded from the blood analysis. All other participants in both groups (high active and low active) did not mention any type of chronic diseases during their participation.

The results of the blood analyses are summarised in Table 4.3.4. The comparison between groups showed that there were no significant differences between groups (high active vs. low active) in most of the blood markers. The only significant differences that were found between groups were in leptin, hsCRP, diastolic blood pressure and mean arterial pressure.

Table 4.3.4 Mean (±SD) and mean differences (95% CI) of blood markers for high active versus low active adults (high active n=22, low active n=25)

Blood markers	High active	Low active	Mean differences (95% CI)
Total Cholesterol (mM)	4.84 ±0.81	4.99 ±1.00	-0.15 (-0.69, 0.39)
High Density Lipoprotein (HDL) (mM)	1.53 ±0.37	1.46 ±0.32	0.06 (-0.14, 0.27)
Very Low Density Lipoprotein (VLDL) (mM)	0.44 ±0.14	0.49 ±0.21	-0.05 (-0.15, 0.06)
Low Density Lipoprotein (LDL) (mM)	2.87 ±0.82	3.04 ±0.88	-0.16 (-0.67, 0.34)
Triglycerides (mM)	0.96 ±0.30	1.06 ±0.45	-0.10 (-0.33, 0.12)
Adiponectin (pg/ml)	6.21 ±3.61	6.60 ±4.67	-0.38 (-2.79, 2.00)
Leptin (pg/ml)	4.51 ±6.13	17.11 ±20.58	-12.60 (-21.82, -3.38)**
Interleukin-6 (IL-6) (pg/ml)	7.21 ±6.40	7.94 ±1.09	0.73 (-6.07, 4.62)
Tumor Necrosis Factor-alpha (TNF-α) (pg/ml)	5.20 ±3.74	4.57 ±2.92	0.63 (-1.33, 2.59)
hsC-reactive Protein (hsCRP) (µg/ml)	1.14 ±1.31	2.19 ±1.94	-1.06 (-2.05, -0.07) *
Insulin (µg/ml)	9.21 ±3.09	8.97 ±2.53	0.24 (-1.41, 1.90)
Glucose (mM)	5.53 ±0.84	5.22 ±0.54	0.31 (-0.11, 0.72)
HOMA-IR	2.26 ±0.84	2.07 ±0.50	0.20 (-0.20, 0.60)
n-6/n-3 (ARA/EPA)	8.16 +4.36	8.04 +3.76	0.12 (-2.35, 2.59)
% n-3 HUFA/Total HUFA	32.47 +9.67	29.29 +8.37	3.19 (-2.31, 8.67)
Blood pressure, Systolic (mmHg)	114.1 ±10.7	119.2 ±11.7	-5.1 (-11.7, 1.6)
Blood pressure, Diastolic (mmHg)	73.5 ±7.0	79.2 ±10.1	-5.8 (-10.9, -0.6) *
Mean Arterial Pressure (MAP) (mmHg)	87.0 ±7.1	92.5 ±10.2	-5.4 (-10.7, -0.2) *

* Differences are significant between groups at P<0.05.

** Differences are significant between groups at P<0.01.

In order to eliminate the effect of the body composition on blood markers, high active lean (n=17) were compared with low active lean (n=12). There were no significant differences observed between groups in any of the blood markers as well as physical parameters except in physical activity levels (MVPA and Vig.&Vvig PA. all *P*<0.01).

4.3.3 Correlations

Pearson correlation was used to examine the associations between variables. Results of the present study indicated that HDL has inversely moderate correlations with weight (r= -0.39 P=0.004) and positive moderate correlation with fat free mass (r=0.39 P=0.003) and a positive correlation with vigorous activity intensity (r=0.27 P=0.049). Moreover, VLDL, also, has correlations with BMI (r=0.33 P=0.041). In fatty acids, %n-3 HUFA was inversely associated with fat free mass (FFM) (r= –0.29, P=0.045) and with the n-6/n-3 ratio (r= –0.85, P=0.000). In addition, n6/n3 ratio was inversely correlated with vigorous and very vigorous physical activity intensity (r= -0.30, P=0.03). On the other hand, adiponectin was correlated with fat free mass and HDL (r= -0.46 and 0.51, both P=0.000 respectively). Leptin has positive correlation with body mass, BMI and BF% (r=0.55, r=0.76 and 0.83 all P=0.00 respectively). High sensitivity C-reactive protein (hsCRP) was positively correlated to some of the body composition. There were moderate to good correlations were found between hsCRP and body weight, BMI and BF% (r=0.36, P=0.01, 0.46 and 0.49 both P=0.00 respectively). Fasting glucose was inversely correlated with percentage time spent in MVPA intensity (r=-0.26, P=0.053).

Table 4.3.5 Pearson correlation between cardiovascular and metabolic disease risk markers and BMI, %BF, % MVPA and % V & Vvig. (*r)*, *P* value.

Blood markers	BMI	% BF	% MVPA	% V & Vvig.PA
Total Cholesterol	(0.14) *P*=0.32	(0.25) *P*=0.06	(-0.18) *P*=0.19	(-0.15) *P*=0.29
HDL	(-0.23) *P*=0.10	(0.02) *P*=0.86	(0.04) *P*=0.80	(0.27) *P*=0.049*
VLDL	(0.33) *P*=0.01*	(0.22) *P*=0.11	(0.14) *P*=0.31	(-0.34) *P*=0.049*
LDL	(0.17) *P*=0.23	(0.22) *P*=0.12	(0.15) *P*=0.28	(-0.23) *P*=0.09
Triglycerides	(0.33) *P*=0.01*	(0.23) *P*=0.11	(-0.14) *P*=0.31	(-0.09) *P*=0.53
Adiponectin	(-0.11) *P*=0.44	(0.26) *P*=0.05	(0.13) *P*=0.34	(0.05) *P*=0.69
Leptin	(0.76) *P*=0.00**	(0.83) *P*=0.00**	(-0.17) *P*=0.24	(-0.8) *P*=0.56
IL-6	(-0.09) *P*=0.51	(-0.04) *P*=0.80	(0.01) *P*=0.97	(-0.08) *P*=0.57
TNF-α	(0.02) *P*=0.87	(0.09) *P*=0.50	(0.06) *P*=0.69	(-0.01) *P*=0.95
hsCRP	(0.46) *P*=0.00**	(0.49) *P*=0.00**	(-0.21) *P*=0.12	(-0.19) *P*=0.17
Insulin	(0.24) *P*=0.07	(0.10) *P*=0.47	(-0.09) *P*=0.53	(-0.15) *P*=0.26
Glucose	(-0.04) *P*=0.77	(-0.01) *P*=0.93	(-0.26) *P*=0.047*	(0.16) *P*=0.24
HOMA-IR	(0.21) *P*=0.12	(0.08) *P*=0.58	(0.05) *P*=0.74	(0.08) *P*=0.58
n-6/n-3 (ARA/EPA)	(-0.04) *P*=0.79	(-0.14) *P*=0.32	(-0.12) *P*=0.39	(-0.30) *P*=0.03*
% n-3 HUFA/Total HUFA	(-0.18) *P*=0.20	(0.03) *P*=0.83	(0.04) *P*=0.78	(0.23) *P*=0.11
BP Systolic	(0.32) *P*=0.02*	(0.26) *P*=0.049*	(-0.13) *P*=0.36	(0.04) *P*=0.75
BP Diastolic	(0.52) *P*=0.00**	(0.41) *P*=0.00**	(0.07) *P*=0.62	(0.04) *P*=0.77
MAP	(0.47) *P*=0.00**	(0.39) *P*=0.00**	(-0.01) *P*=0.97	(0.04) *P*=0.75

BMI: body mass index, %BF: percentage body fat, MVPA: moderate to vigorous physical activity, V & Vvig.: vigorous and very vigorous physical activity, BP: blood pressure, MAP: mean arterial pressure.

* Indicates significant correlation at P<0.05

** Indicates significant correlation at P<0.01

Overweight/obese and low active participants were more likely to be at risk of cardiovascular and metabolic diseases than lean and high active participants. These results are indicated in Table 4.3.6.

Table 4.3.6 Number of participants who may be at risk of metabolic syndrome:

	NCEP-ATPIII Metabolic syndromes	3 Risk markers	4 Risk markers	5 Risk markers	≥6 Risk markers
Lean	0	4	4	2	1
Overweigh/obese	5	3	5	3	9
High active	1	6	2	1	4
Low active	3	3	0	4	7

4.4 Discussion:

4.4.1 Association between body composition and cardiovascular metabolic risk factors and in adults.

Physical characteristics of the fifty five participants presented in Table 4.3.1 showed significant differences in body composition between lean versus overweight/obese groups. The association between cardiovascular and metabolic risk factors and obesity has been investigated previously (Jakicic *et al.*, 1993; Cho *et al.*, 2009; Rokling-Andersen *et al.*, 2007). For instance, cardiovascular disease risk markers are more likely to be higher in overweight/obese adults comparing to lean adults (Jakicic *et al.*, 1993; Cho *et al.*, 2009; Rokling-Andersen *et al.*, 2007). However, fit obese adults have been found to have lower morbidity and mortality than sedentary normal weight adults (Blair and Brodney, 1999).

The increased prevalence of obesity among adults with hypertension and hypertension among the obese adults has also been investigated (Kannel *et al.*, 1967; Stamler *et al.*, 1978; Blair *et al.*, 1984). In addition, high blood pressure is

associated with cardiovascular diseases independently (Tanomsup *et al.*, 2007). The blood pressure parameters in the present study, including (systolic, diastolic and mean arterial pressure (MAP)) between lean and overweight/obese groups were significantly different. However, all blood pressure values in both groups were within normal range, and this may affect the risk associated with other factors such as physical activity status. Most of the overweight/obese participants in the present study were at least low active but not sedentary. Particularly, more than 22.5% of them were highly active, which may play a role in keeping blood pressure of the overweight/obese group within normal range.

In the present study, the traditional cardiovascular disease risk markers (blood lipids) were significantly higher in overweight/obese comparing to the lean group except HDL as it was significantly lower in overweight/obese group. The results of the current study found that both groups had normal concentration levels of triglyceride, HDL and fasting glucose. However, the lean group reported normal concentrations of total cholesterol, LDL and VLDL whereas overweight/obese group was at the borderline risk and significantly higher than lean's levels ($P<0.05$, $P<0.01$and $P<0.01$ respectively). Based on the updated guidelines of the National Cholesterol Education Programme, Adults Treatment Panel III (NCEP-ATP III) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001), diagnosis of metabolic syndrome can occur if three of the following cardiovascular risk factors are present: 1) waist circumference $\geq$102cm for men, $\geq$88cm for women; 2) triglyceride level $\geq$150mg/dL ($\geq$2.3mM/L); 3) HDL level <40mg/dL (<1.0mM/L)

for men and <50mg/dL (<1.3mM/L) for women; 4) blood pressure ≥130/85 mmHg; 5) fasting glucose ≥110mg/dL (5.5mM/L). Similar guidelines of cardiovascular and metabolic risk markers have been issued by the Clinical Chemistry Service Handbook (CCSH) published by North Glasgow University Hospitals Division (Clinical Chemistry Service Handbook, 2010). In the present study, Table 4.3.6 indicates that 5 of the overweight/obese participants at risk of metabolic syndromes as they had 3 or more of the cardiovascular risk markers whereas none of the lean participants had 3 or more of the cardiovascular risk markers. In addition, 9 of the overweight/obese participants had 6 or more of the cardiovascular and metabolic disease risk markers whereas only 1 had 6 or more of these risk markers in the lean group.

Parallel with the previous evidences (Brown *et al.*, 2007; Cho *et al.*, 2009), the present study confirms that overweight and obese adults have elevated blood lipids (total cholesterol, VLDL and LDL) which may contribute to development of cardiovascular diseases (Epstein *et al.*, 1976; Morris *et al.*, 1953; Cho *et al.*, 2009; Sung *et al.*, 2007). However, there were no significant differences between groups in highly unsaturated fatty acid profiles, with both groups showing low n-3/total HUFA percentage which is largely reflective of a dietary intake low in n-3 oils (Marangoni *et al.*, 2007). In addition to the physical activity status, the risk of the negative changes in the blood lipids concentrations (elevated total cholesterol, triglyceride, LDL and VLDL, and decreased HDL) may be related to some other factors such as diet.

The balance of omega 3 and omega 6 in highly unsaturated fatty acids (HUFA) is an important index for cardiovascular diseases (Lands, 2003; Thorseng *et al.*,

2009). Based on the published criteria of n-6/n-3 (ARA/EPA) for UK adults (ARA/EPA= ~16) as an inflammation marker (Bell *et al.*, 2010), the participants of the current study were within normal range (Lean=7.7, overweight/obese=8.3). However, most of the participants had similar or higher values of %n-3 HUFA/total HUFA comparing to the observed values for healthy adults who consuming low and high portions of fish per week (low ≤1 portion/week, high >1portion/week) (Marangoni *et al.*, 2007), which were between 24.6 and 27.4 respectively, whereas the values in the present study were (lean=32.0, overweight/obese=28.5).

On the other hand, Ideal Omega Test web site provides a scale of the % omega 3 healthy values as follows: 20% take urgent remedial action, 30% take remedial action, 40% remedial action beneficial, 50% healthy omega 3 level and 70% perfect for optimal level (Ideal Omega test (no publication date). Available: http://www.idealomegatest.com). The participants in the current study in both groups may consume diet containing omega 3 but they maybe need to increase the amount of omega 3 and reduce omega 6 for cardiovascular disease prevention.

Body composition may also play a role in the concentrations of the, relatively, new cardiovascular and metabolic risk markers such as adipokines / inflammatory cytokines.

Adiponectin is known as a hormone induced by adipose tissue and found to enhance insulin sensitivity and increase free fatty acid (FFA) oxidation in several tissues including muscle tissues (Fruebis *et al.*, 2001; Yamauchi *et al.*, 2001; Scherer *et al.*, 1995). It has been demonstrated that low level of

adiponectin concentrations is correlated with insulin resistance and hyperinsulinemia as well as coronary artery disease (CAD) (Hotta *et al.*, 2000; Hotta *et al.*, 2001). Moreover, one of the major factors that have been found to play a role in adiponectin concentration is the body fat (Kondo *et al.*, 2006; Arita *et al.*, 1999). In the current study, adiponectin concentrations were not significantly different between lean and overweight/obese groups. Furthermore, adiponectin concentrations in both groups were within normal range but plasma adiponectin was found to be negatively correlated to fat free mass (FFM) (r=0.46, P=0.000). However, this correlation might be due to the number of overweight/obese females. In a recent study, Jürimäea J. *et al.* (2009) found that adiponectin was related to FFM in overweight women (*P*=0.000) but not in normal-weight women (Jürimäea *et al.*, 2009).

Regardless of body adiposity, women tend to have higher circulating adiponectin than men (Spranger *et al.*, 2003). Similar finding was observed in the present study. When lean men (n=15) are compared with overweight/obese men (n=12) in the current study, adiponectin concentration was not significantly different (*P*=0.10). Similar results were found between lean women (n=18) versus overweight/obese women (n=10) (*P*=0.85). However, there was a strong significant differences in adiponectin concentrations between men (n=27) and women (n=28) regardless of the body fat percentage (*P*<0.000). The present study demonstrated that adiponectin concentration may be affected by gender more than body fat percentage. This result may refer to the nature of the lower lean mass (fat free mass) in female compare to the male. In terms of normal range, the current study demonstrated that adiponectin concentration was not

affected by overweight/obesity with low physical activity –but not sedentary- in apparently healthy adults. Moreover, the negative effect of body fat percentage on adiponectin concentrations may appear in high level of obesity or in other factors such as sedentary lifestyle.

In contrast, the present study showed that plasma leptin concentration seems to be affected by body composition (body adiposity). The data of the current study indicated that plasma leptin concentrations were significantly higher in overweight/obese group compared to the lean group (P<0.01). These results agreed with the previous studies as plasma leptin concentrations found to be correlated with BMI (Maffei *et al.*, 1995) and significantly higher in obese adults comparing to lean adults (Greenberg and Obin, 2006). Moreover, plasma leptin concentrations were found to be high in most of the obese adults because of the increased amount of leptin-secreting adipose tissue (Considine *et al.*, 1996).

On the other hand, plasma leptin has been found to be associated with inflammation and in particular to CRP, a blood marker of low-grade inflammation strongly related to obesity and a predictor for coronary heart disease (CHD) (Shamsuzzaman *et al.*, 2004; Maachi *et al.*, 2004; Keller *et al.*, 2003). The present study showed high correlation between plasma leptin concentration and body weight, BMI and Body fat% (P<0.000). Thus, supporting previous evidence that increasing body adiposity may contribute to developing the risk of cardiovascular and metabolic diseases even in apparently healthy overweight/obese adults with low to moderate physical activity. This is likely to be relevant when it is elevated with other related

cardiovascular and metabolic risk markers such as hsCRP, LDL and VLDL even in apparently healthy non-sedentary overweight/obese adults. The present study suggested that plasma leptin concentration could be a sign of the earlier cardiovascular risk marker. Thus, overweight/obese adults may need to be involved in an adequate physical activity programme for the purpose of weight loose in order to help for regulating plasma leptin concentration.

C-reactive protein (CRP) is recognised as a marker of inflammation and a major and independent predictor of cardiovascular disease risk marker and has been shown to be elevated in obese adults (Lindmark *et al.*, 2001; Ridker *et al.*, 2002). The results of the current study found that high-sensitivity C-reactive protein (hsCRP) concentration was influenced by body composition significantly ($P<0.01$). This is not a surprising finding as several studies have demonstrated that hsCRP was affected by body composition more than other factors such as physical activity status (Monzillo *et al.*, 2003). For example, Monzillo *et al.* (2003) examined the effect of lifestyle modifications on adipokine level in obese adults. Although a significant reduction was found in the body weight and BMI after an exercise programme ($P=0.02$, $P=0.00$ respectively), hsCRP in the apparently healthy obese adults was not influenced ($P=0.49$) (Monzillo *et al.*, 2003). One of the explanation is that the participants were still in obese status (BMI=34.3$\pm$1.0). Ridker and colleagues (2001) has developed hsCRP risk range obtained from 5000 apparently healthy adults and suggested that $\geq$2.0ug/ml of hsCRP is considered to be high. They suggested that elevated CRP with high ratio of total cholesterol:high density lipoprotein cholesterol (TC:HDL-C) represent a very high cardiovascular risk marker for both male and

female (Ridker *et al.*, 2001). In the current study, the overweight/obese group has significantly higher hsCRP (2.64±0.42ug/ml) and higher TC:HDL-C comparing to lean group (1.13±0.21ug/ml). Accordingly, overweight/obese participants are probably at risk of future cardiovascular and metabolic disease more than the lean participants. In addition, elevated hsCRP concentration has been found to be associated with insulin resistance (Lemieux *et al.*, 2001). Similar results were found in the present study, as the overweight/obese group had high levels of insulin and HOMA-IR (10.29 ±0.78 and 2.45 ±0.19, respectively, both $P<0.01$). The current study demonstrated that obesity may play a role in elevating some of the important and newly risk markers such as hsCRP which considered as independent cardiovascular and metabolic risk markers. Furthermore, hsCRP may be considered as an early risk marker beside leptin and some of the blood lipids markers. In addition, the elevation in hsCRP has occurred in apparently healthy overweight/obese group whom were not sedentary.

The present study reconfirms that obesity itself causes negative changes in some of the cardiovascular and metabolic disease risk markers even in low active overweight/obese adults which, in turn, contribute in developing cardiovascular and metabolic diseases independent of their physical activity status.

Insulin is well known as a hormone secreted by the pancreas in response to food intake. Abnormal increase in fasting insulin level or in response to feeding in the blood serum may cause insulin resistance, which may result in type 2 diabetes. Data of the current study showed that overweight/obese group had

significantly higher fasting insulin level than lean group. However, both groups were below the risk level (<13mU/L). Although overweight/obese group did not exceed the risk border of the fasting insulin level, they may be at risk especially when other cardiovascular and metabolic risk markers were in high concentrations. For instance, leptin and hsCRP concentrations in the overweight/obese group were high in the present study.

On the other hand, fasting glucose levels of both groups were within normal range and not significantly different. However, overweight/obese in the present study may have diet restriction and they were doing some physical activity. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated in the current study to assess the whole-body insulin resistance. Data showed that HOMA-IR was significantly higher in overweight/obese group than lean group ($P<0.01$). The normal values for HOMA-IR are still under discussion and ranged between 2.0 - 2.5 (Kashiwabara *et al.*, 2000; Taniguchi *et al.*, 2000). When the published of HOMA-IR cut-off is used to assess insulin resistance; overweight/obese group was very close to the risk threshold value. This result may suggest that the overweight/obese participants were at risk of having insulin resistance more than lean participants even though they were quite active.

Data of the present study found that there were no significant differences between lean versus overweight/obese groups in plasma IL-6 concentrations (P=0.95). Both lean and overweight/obese groups were within normal range of plasma IL-6 concentration (0.6-20pg/ml) (Morabito *et al.*, 2007). It has been demonstrated that IL-6 secreted from subcutaneous adipose tissue was

positively correlated with BMI and percent body fat (Kern *et al.*, 2001). As mentioned previously, IL-6 is secreted by different tissues and its plasma concentrations can be influenced by many different factors. For example, nutrient availability such as carbohydrate ingestion may blunt IL-6 production (Keller *et al.*, 2003). On the other hand, it has been demonstrated that plasma IL-6 concentration decreased significantly after body weight reduction in obese adults (Monzillo *et al.*, 2003; Bastard *et al.*, 2000; Ziccardi *et al.*, 2002). However, the absence of the association between plasma IL-6 concentrations and body composition in the present study showed that it is possibly affected by other factors such as physical activity status as all participants were either high active or low active including overweigh/obese participants. Although plasma IL-6 concentrations has been found to play a role in some of the cardiovascular and metabolic diseases, negative changes in IL-6 levels however may not be affected directly by obesity itself. Therefore more investigations are needed to examine the effect of body composition on plasma IL-6 concentration in apparently healthy adults.

Almost all adipose tissue TNF-α expression is secreted by macrophages (Weisberg *et al.*, 2003) and plasma TNF-α concentration has been identified as myocardial infarction risk predictor (Reilly *et al.*, 2007). In the present study, no significant differences were found between lean and overweight/obese groups in plasma TNF-α concentration. Although a previous study has showed that TNF-α concentration is elevated in obese adults (Hotamisligil *et al.*, 1995), others have demonstrated that the association between TNF-α concentration and obesity is not clearly understood (Berggren *et al.*, 2005). The effect of

obesity on elevated TNF-α could be indirect (Berggren *et al.*, 2005). Plasma TNF-α, as with IL-6, is found to be influenced by different factors such as diet and physical exercise (Hayase *et al.*, 2002). Thus, plasma TNF-α concentration probably plays a role as a chronic risk marker in some of the metabolic diseases such as type 2 diabetes but not as an acute risk marker. The association between plasma TNF-α concentrations and body composition may need more investigations in order to clarify the influence of obesity as an independent factor on the TNF-α concentration.

4.4.2. Association between physical activity status and cardiovascular and metabolic risk factors in adults.

The association between cardiovascular and metabolic risk markers and high versus low physical activity status in apparently healthy adults has been investigated in the present study. Physical characteristics of the forty seven participants who met the physical activity criteria show significant differences in MVPA and predicted VO_{2max} ($ml.kg^{-1}.min^{-1}$) between high active versus low active groups (Table 4.3.3). Physical activity status is one of the major factors that play a role in number of the cardiovascular and metabolic diseases risk markers. Blair and Brodney (1999) concluded that active obese individuals have lower morbidity and mortality than normal weight individuals who are sedentary. They also, added that physical inactivity and low cardiorespiratory fitness are as important as overweight and obesity as mortality predictors. The effect of the physical activity status (low active vs. high active) on some of the traditional and the new discovered cardiovascular and metabolic risk markers

have been investigated in the present study using objective measurement method (see Chapter 3 for more detail about physical activity assessment using accelerometry device).

Data of the current study showed that blood pressure was associated with physical activity status. Results of the present study showed that both diastolic and MAP were significantly lower in high physical activity group than low active group. Although the blood pressure parameters observed in low active group were within normal range, the current study demonstrated that adults with high physical activity showed heather blood pressure status comparing to low active adults.

The effect of high versus low physical activity status on the classical cardiovascular disease risk markers was investigated in the present study. The results indicated that there were no significant differences between high active group and low active group in total cholesterol, VLDL, LDL, HDL or triglyceride. Furthermore, both groups showed normal levels in most of the traditional cardiovascular disease risk markers (total cholesterol, HDL, triglyceride). However, low active group was at the borderline risk of LDL concentration. The effect of physical activity status and exercise on the cardiovascular disease risk markers has been examined in number of previous studies (Polak *et al.*, 2006). Polak and colleagues (2006) studied the effect of short-term physical training (3 months) at moderate intensity on plasma level of some adipokines and blood lipids markers for obese women. Although the short-term physical training did significant changes in body weight, BMI and BF% (P<0.01), there were no significant changes in total cholesterol, HDL, triglyceride or fasting glucose

levels (Polak *et al.*, 2006). According to the NCEP-ATP III criteria (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001), the participants in Polak's study were within risk zone even after short-term of physical training session. The influence of the physical activity as a health factor may appear in long term physical training. Polak and colleagues referred the results to the type and duration of the training session as well as the sex and degree of obesity of the participants (Polak *et al.*, 2006).

The effect of exercise training on blood lipids for obese women – but not for the lean women– may be associated with high physical exercise intensity (Kondo *et al.*, 2006). In a review study by Williams PT (2001), intervention studies showed that runners who were doing high intensity exercise had changes in HDL that were strongly dependent upon loss of weight. As discussed previously, cardiovascular disease risk markers increase in adults with increase in high body fat. Thus, blood lipids concentrations as classical cardiovascular disease risk markers may be affected by adiposity more than physical activity especially in normal weight adults. However, exercise intensity may play a role in the positive changes similar to what has been found in some studies (Kondo *et al.*, 2006). Therefore, the current study showed that the traditional cardiovascular risk markers (blood lipids) appeared between lean versus overweight/obese groups more than between low versus high physical activity status. This result could be explained by the number of lean participants in low active group as they were about half of the low active group (12 lean, 13 overweigh/obese) whereas there were only 5 overweight and 17 lean in the high active group. To insure that this result affected by the body composition and not by the physical

activity status, high active lean (n=17) and low active lean (n=12) were compared.

The results showed that both groups had normal levels in all traditional blood lipids markers (total cholesterol, VLDL, LDL, HDL and triglyceride) and there were no significant differences existed between groups (high active lean vs. low active lean). However, a correlation between vigorous physical activity and HDL was found ($r=0.27$, $P<0.05$). High active group showed significantly higher vigorous physical activity than low active group and higher HDL but this was not significant. In conclusion, the low active group in the present study showed an average of 141 minutes of moderate to vigorous physical activity per week and this amount of physical activity may be help them to keep their blood lipids components (total cholesterol, VLDL, LDL, HDL and triglyceride) within normal level except in LDL level as the data showed that their LDL was at the borderline of the risk level. Furthermore, lean participants with low physical activity had acceptable levels of all blood lipids markers.

The data of the current study may suggest that in the condition of apparently healthy lean adults, about 141 minutes of MVPA may be enough to keep blood lipids markers within recommended levels. However, performing MVPA within recommended guidelines ($\geq$150mins/w) and keeping body fat within normal range is likely to improve blood lipids concentrations positively.

In terms of fatty acid index, the comparison between high active versus low active showed similar results were found between lean versus overweight/obese groups which may be referred to diet more than physical activity influence. However, there were a positive moderate correlation between

omega 3 levels and vigorous physical activity. This means that physical activity at high intensity levels may enhance the utility of the consumed foods contain omega 3. Other explanation is that active people in the present study may have –relatively– more healthy diet including food with rich of omega 3 and low of omega 6. Although there were no significant differences between high active versus low active groups in fatty acids indexes (n-6/n-3 and %n-3 HUFA/total HUFA), physical activity intensity or exercise training may have an indirect influence on the usage of the consumed fatty acids including omega 3. More investigation may be needed to clarify the association between physical activity and fatty acids.

Adiponectin and leptin; as newer cardiovascular and metabolic risk markers, have been found to be affected by physical activity and exercise (Blüher *et al.*, 2006; Kondo *et al.*, 2006; Rokling-Andersen *et al.*, 2007). In the present study, there was no significant difference detected in adiponectin concentrations between low versus high physical activity groups. Low active group had higher -but not significant- adiponectin than high active group. The present result agreed with a previous study (Polak *et al.*, 2006). Exercise training programme (~225mins/wk for 3 months at 50% of VO_{2max}) did not influence adiponectin concentrations (Polak *et al.*, 2006). However, long-term of endurance physical exercise with moderate intensity may increase adiponectin level (Kondo *et al.*, 2006). Kondo and colleagues (2006) investigated the effect of exercise on circulating adipokines including adiponectin in obese young women. They found that after 7 months of endurance exercise at 60-70% of $HR_{max\ reserved}$ for about 200min/w, adiponectin level increased significantly in obese group

(*P*<0.01), but not in lean women (Kondo *et al.*, 2006). This is probably due to the weight loss in obese women. To eliminate the effect of body composition between low and high active groups in the present study, only lean participants were compared. The differences in adiponectin concentrations did not appear significantly even between lean low active participants (n=12) versus lean high active participants (n=17) (*P*=0.95). In addition, both groups have normal adiponectin concentrations. Gender effect was examined among low and high active groups.

Regardless of physical activity status, lean female participants (n=17) have significantly higher adiponectin concentrations than lean male participants (n=12) (*P*<0.000). The effect of physical activity status and exercise training on plasma adiponectin concentration is not fully clear. Thus, more studies may be needed to identify the influence of physical activity status on circulated adiponectin in apparently healthy adults.

On the other hand, data of the present study indicated that low active group has significantly higher plasma leptin than high active group (*P*<0.01). The effect of exercise and physical activity level on plasma leptin as cardiovascular disease risk marker has been examined in several studies (Wannamethee *et al.*, 2007; Berggren *et al.*, 2005; Polak *et al.*, 2006; Monzillo *et al.*, 2003; Jürimäea *et al.*, 2009). Duration and intensity of physical activity training may play a role in the plasma leptin concentrations. For example, long-duration of exercise training (Perusse *et al.*, 1997) and sufficient high intensity of physical activity (Elias *et al.*, 2000) have been shown to decrease the circulating leptin concentrations in adults independently. In contrast, short-term exercise training did not influence

fasting plasma leptin concentration (Houmard *et al.*, 2000). The influence of long-term exercise training has been related to the body weight reduction (Christensen *et al.*, 1998). However, several studies have demonstrated that the effect of the long-term exercise training was independent of weight reduction (Hickey *et al.*, 1997).

Supporting the previous studies (Elias *et al.*, 2000), high active group in the present study has spent vigorous and very vigorous physical activity significantly more than low active group (P=0.01). Partly, this may explain why high active group have significant lower level of plasma leptin comparing to the low active group. However, this differences did not appear between lean participants (high (n=17) versus low (n=12) active groups) (P<0.18) and both groups were within normal values. In general, high physical activity (MVPA $\geq$300mins/wk) may influence the plasma leptin concentrations positively and in turn, may contribute to reduce the risk of cardiovascular and metabolic diseases. Yet, it is not fully clear whether or not low physical activity lifestyle (MVPA <150mins/w) is enough to influence plasma leptin concentrations positively especially in apparently lean healthy adults. Further studies are suggested to examine whether or not low physical activity level can contribute in any positive changes in plasma leptin concentrations.

C-reactive protein (CRP) is an independent predictor of cardiovascular disease risk marker and an important marker of inflammation (Danesh *et al.*, 2004; Lindmark *et al.*, 2001; Ridker *et al.*, 2002). In the present study, high active group showed significantly lower hsCRP concentrations than low active group (P<0.05). However, the differences found between high active versus low

active groups were possibly affected by the number of the overweight/obese participants as they were 52% of the low active group whereas only 22.7% of active group were overweight/obese participants. However, other factors such as physical activity intensity may play a role on hsCRP concentration. In a recent study, Andersson *et al.* (2010) examined the effects of heavy endurance physical exercise on inflammatory markers in non-athlete lean adults. They found that CRP was decreased significantly during the recovery period (the 8th week) following the high intensity physical exercise programme.

Therefore, high intensity of physical activity may reduce the CRP concentration even in lean adult with normal concentrations (Andersson *et al.*, 2010; Mattusch *et al.*, 2000). As mentioned previously, lean high active group showed significantly higher amount of time spent in vigorous and very vigorous than lean low active group in the present study. Although no significant differences existed in hsCRP concentrations between the groups (P=0.27), yet both groups were within normal range. Similar to leptin, low physical activity (about 141mins/w) might be enough for the apparently healthy lean adults to maintain hsCRP concentrations at the normal level. Moreover, there were strong correlations between hsCRP and body weight, BMI and BF% as well as with leptin (r=0.36 P=0.008, r=0.46 P=0.000, r=0.49 P=0.000 and r=0.34 P=0.013 respectively) but not with any of physical activity levels. More focus researches may be needed to investigate the role of physical activity status in hsCRP concentrations in apparently healthy adults with considering gender effect.

Acute physical exercise has been found to be one of the factors that play a role in plasma IL-6 concentrations (Ullum *et al.*, 1994; Ostrowski *et al.*, 1998; Drenth

et al., 1995). However, other studies failed to find significant differences in plasma IL-6 concentrations between pre and post exercise training programme (Polak *et al.*, 2006; Fischer *et al.*, 2004). The present study showed that although high active group showed relatively lower value of plasma IL-6 than low active group, plasma IL-6 concentrations were not significantly different between low active versus high active adults even between lean participants in each group. However, plasma IL-6 concentrations were within normal range in both groups (normal range 0.6 – 20pg/ml) (Morabito *et al.*, 2007). The influence of physical activity status on IL-6 is still vague and the effect of physical activity status seems to be not independent of other factors such as weight reduction (Monzillo *et al.*, 2003).

It has been suggested that more investigations about the effect of physical activity status on plasma IL-6 concentrations in healthy adults are required (Polak *et al.*, 2006). On the other hand, the current study found good correlations between plasma IL-6 and VLDL and triglyceride (r=0.42, *P*=0.001 in both). Therefore, plasma IL-6 concentrations may not be an acute marker of predicting some of the cardiovascular and metabolic disease in apparently healthy adults with low physical active status. Partly, because the plasma IL-6 concentrations in low physical active group was within normal range and was not significantly different from the high physical active group whereas some other acute newly discovered cardiovascular and metabolic risk markers such as leptin and hsCRP showed significant differences between groups.

Similar to the plasma IL-6 results, Tumor Necrosis Factor- alpha (TNF-α) did not show significant differences between low active versus high active groups

in the present study. Plasma TNF-α has been investigated under the effect of physical exercise (Sloan *et al.*, 2007; Andersson *et al.*, 2010; Polak *et al.*, 2006). Moderate exercise training programme has shown no effect on the circulated TNF-α concentration (Polak *et al.*, 2006; Sloan *et al.*, 2007), whereas 12 weeks of high exercise intensity has resulted in significant reduction in inducible TNF-α synthesis in whole blood in healthy young and sedentary adults (Andersson *et al.*, 2010). However, the influence of high intensity of exercise training has been found to be temporary. In a recent study by Andersson and colleagues (2010), they found that heavy exercise training has increased TNF-α significantly in fit men -but not athletic- in the first and second weeks of the training. Then the TNF-α concentrations reduced to the baseline level during the recovery period time of the sixth and eighth weeks. In the current study, high active group spent more time in vigorous and very vigorous activity than low active group significantly. However, there is no significant difference between them in TNF-α concentration. Moreover, the results of the present study confirm that there is no significant correlation between TNF-α and BMI or weight which has been shown by other studies (Sloan *et al.*, 2007; Andersson *et al.*, 2010). Thus, the role of physical activity on the circulated TNF-α as an anti-inflammatory marker possibly be related to indirect mechanisms. More investigations are needed in order to fully understand the role of physical activity status on the TNF-α concentration.

There were no significant differences between low active versus high active groups in fasting insulin, glucose or HOMA-IR in the present study. Surprisingly, High active group were at the borderline of the upper level of

fasting glucose concentration and both groups were at the borderline of high level of HOMA-IR ratio. This result may be partly related to a couple of the participants in the high active group as they had very high level of fasting glucose (~7.0mM). However, the averages of all values were within normal range. Physical activity status plays a role in reduce and regulate plasma leptin concentrations. In the same time physical activity is a strong determinant of insulin sensitivity with or without obesity (Kelley and Goodpaster, 1999). Several studies demonstrated that leptin acts as an insulin sensitizer in the case leptin concentrations are at low or normal levels and may contribute to insulin resistance when leptin is constantly elevated (Franks *et al.*, 2007). The results of the present study demonstrated that in apparently healthy lean adults; low level of physical activity may be beneficial in keeping insulin and glucose as well as HOMA-IR within normal levels.

Although the sample size was almost close to the required number for each group (30 participants in each group at power of 80%), only fifty five participants were able to provide a blood sample. Due to the time limit given to complete the current study, the sample size of the current study was somewhat below the number required to generalise the results especially if the power calculation to be used was increased to 90% as the sample size would then be required to reach 42 in each group (lean vs. overweight/obese).

4.5 Conclusion

Results of the present study can be summarized by tables 4.3.5 and 4.3.6. The tables show the number of participants who had 3 or more of the cardiovascular and metabolic risk markers. Based on the NCEP-ATPIII, overweight/obese and low active participants had more chance of having cardiovascular diseases than lean and high active participants.

On the other hand, a number of the other risk markers, including the newer risk markers, were higher in overweight/obese and low active participants especially when the number of the risk markers was more than 3. Thus, both body composition and physical activity status play independently and integrated roles in most of the traditional and some of the newer discovered cardiovascular and metabolic diseases risk markers. Overweight and obesity may contribute in developing cardiovascular and metabolic disease even in low active adults stressing the importance of meeting the physical activity guidelines. The risk is reduced in high physical activity status ($\geq$300mins/w) regardless of adiposity. Moreover, most of the participants were young adults (<40yr) which adds further weight to the importance of controlling adiposity and increasing physical activity in this age group if future morbidity and mortality rates are to be reduced. The interaction of physical activity and adiposity on risk markers requires further study to define strategies that can reduce cardiovascular and metabolic diseases, but the data from the present work suggest that higher levels of physical activity and particularly moderate, vigorous and very vigorous activity are associated with a reduction of risk markers.

Chapter 5. Habitual physical activity assessment in Scottish adolescents using accelerometry and heart rate monitoring.

5.1 Introduction:

Habitual physical activity assessment is important because being physically inactive may cause a number of chronic diseases (Epstein *et al.*, 1976; Morris *et al.*, 1953; Morris *et al.*, 1990; Panagiotakos *et al.*, 2003; Fang *et al.*, 2003). A number of chronic diseases such as heart disease, osteoporosis and some other diseases that appear in adulthood, in fact, are developed in childhood and adolescence (Parsons *et al.*, 1999).

In the previous chapter of this thesis the importance of high physical activity intensity as well as low adiposity were revealed as the key factors influencing risk of disease. However, the health benefits of physical activity to young people are not as well investigated as they are in adults (Currie *et al.*, 2004). Strong and colleagues (2005) systemically reviewed 850 articles to review the effects of habitual physical activity on health and to develop evidence-based recommendations for physical activity in youth. They concluded that one hour or more per day of appropriate and enjoyable moderate to vigorous physical activity is necessary to achieve the desired health and behavioural beneficial outcomes in youth age (Strong *et al*., 2005). Physical activity in early age such as adolescence may help to improve adoption of a healthy lifestyle in adulthood and may reduce chronic disease such as some of the cardiovascular and metabolic diseases (Hallal *et al.*, 2006) and can carry over into adulthood as

they are more likely to be more active when they reach adulthood (Currie *et al.*, 2004). Moreover, a recent study by Martins *et al.* (2010) found that regardless of adiposity, adolescents with higher cardiorespiratory fitness were at lower prevalence of cardiovascular disease risk factors. Therefore, an adequate improvement in physical activity must start at an early age such as childhood (Morris *et al.*, 1953).

This previous evidence raises the importance of assessing physical activity in children and adolescents as a step for investigating the relationship between physical activity status and cardiovascular and metabolic disease risk markers in order to improve and adequately recommend physical activity guidelines for adolescents. However, physical activity assessment for children and adolescents is difficult (Armstrong and Welsman, 2006; Bringolf-Isler *et al.*, 2009).

The habitual physical activity in adolescents should be assessed objectively using an accurate and valid measure. Accelerometry as an objective measure such as Actigraph (AG) has been found to produce valid and reliable habitual physical activity assessment in children and adolescents (Puyau et al., 2002; de Vries et al., 2010) and is deemed accurate for assessing adolescents' physical activity in large epidemiological studies (Hallal et al., 2006). In a systemic review study that researched the quality of movement sensing devices used to assess physical activity in healthy children and adolescents (2-18yr), AG was found to be the most reliable and valid device for this group (de Vries *et al.*, 2006; de Vries *et al*. 2010).

Physical activity status of adolescents has been assessed previously for the purpose of improving public health policy (Riddoch *et al.*, 2004). Parallel to the recent studies in some of European countries, the present study, as a cross-sectional study, has taken place in order to contribute to other studies to build up a clear image about the habitual physical activity status for adolescents in Scotland using valid and reliable objective measures (accelerometer and HR monitor). Thus, an accurate assessment of the adolescent's physical activity in relation with health risk factors such as cardiovascular and metabolic disease is needed (Steene-Johannessen *et al.*, 2010, Watts *et al.*, 2005).

5.1.1 Aims of the study

- To assess the habitual physical activity status for apparently healthy male and females Scottish adolescents (12-17yrs) using an Actigraph accelerometer (GT1M) and HR monitor.
- To compare the physical activity data of the adolescents in the present study to similar data of adolescents in different countries.

5.2 Materials and methodology

5.2.1 Subjects

A total of 34 apparently healthy adolescents aged 12 -17 years old (males n= 17, females n= 17) participated in the present study. In addition to the enhanced disclosure; successful approval for the present study was obtained from the research ethics committee of the University of Stirling and from NHS Forth Valley and Fife. Thirty two of the total participants (males n= 16, females

n= 16) completed their participation in the present study. There were two participants (male n=1, female n=1) who could not complete their participation for personal reasons. From the thirty two volunteers who participated, only one boy and two girls were classified as obese (100^{th}, 99^{th} and 98^{th} percentile for age respectively). The sample size of the present study did not reach the required number of participants in each group (male vs. female) as the power calculation indicated that at power of 80% the required number of participants is 28 participants in each group. However, the number of participants would have to be increased to 48 participants if the power calculation for the sample size was based on a power of 90%. Due to the time limit to complete the current study, the present study must only be considered to be a first step for further investigations. Physical characteristics of the participants are presented in Table 5.1.

Table 5.1 Physical characteristics presented as means ±SD for 32 adolescents (12-17yr), (males n= 16, females n= 16).

Physical characteristics	**All adolescents**	**Male**	**Female**
Age (yrs)	14.3 ±1.6	14.6 ±1.8	14.0 ±1.5
Mass (Kg)	58.5 ±12.7	58.8 ±15.7	58.1 ±9.4
Height (cm)	166.5 ±10.2	169.6 ±12.7	163.3 ±5.7
BMI (Kg.m-2)	21.1 ±4.3	20.2 ±4.7	22.1 ±3.7
BMI Percentile for age	60^{th} ±29^{th}	51^{st} ±27^{th}	68^{th} ±29^{th}
Body Fat%	20.4 ±10.1	14.4 ±10.2	26.4 ±5.2
Fat Free Mass (Kg)	46.0 ±8.8	49.6 ±10.3	42.4 ±4.9
Mean arterial pressure (mmHg)	82.9 ±8.9	83.7 ±8.3	82.0 ±9.7
Resting heart rate (bpm)	66.6 ±11.4	62.1 ±11.7	71.1 ±9.5

Predicted VO_{2max} ($L.min^{-1}$)	2.8 ±0.9	3.1 ±1.1	2.6 ±0.7

All participants were recruited from local high schools as well as local public areas such as sport gyms in Stirling city. All participants with their parents were informed about the study by an invitation letter, poster or leaflet. Subsequently, interested participants who would like to take part in the study contacted researchers via email, letter or telephone. Then they were provided with an information sheet describing the study in detail. If they decided to take part in the study, an initial briefing visit (first visit) was arranged with them (the child with his/her parent/legal guardian) to explain all parts and stages of the study.

5.2.2 Study design

5.2.2.1 Anthropometric and body composition

Participants attended the research science laboratory at the University of Stirling with at least one guardian adult (usually with their parent(s)) for the first visit. During the first visit, participants were given time (10-15 minutes) to have a rest by setting on a chair while the procedure of the study was explained including how to use the devices (Actigraph (AG), and heart rate monitor (HR)) and Figure 5.1 illustrates how they were instructed to wear the devices. Any questions were answered. Then, if they were still happy to participate, the study consent form was signed by both the participant and his/her guardian (parent) and then signed by the principal investigator. After that, body measurements were taken including blood pressure, resting heart

rate, weight, height, and body fat percentage. Body mass was measured in Kilograms to the nearest 50 grams and height was measured in centimetres to the nearest 2 millimetres. Then body mass index (BMI) (body mass(Kg) x height(m^2) was calculated and then converted to a BMI centiles using UK 1990 reference chart for children and adolescents (Cole *et al.*, 1995). Percentage body fat (%BF) was estimated using foot to foot bioelectric impedance analysis. Participants were classified as lean when their BMI <85th centile for age and as obese when their MBI $\geq$98th centile for age.

5.2.2.2 Instruments and physical activity measurement

Table 5-2 Details of the instrumentations used in the present study.

Measurements	Name and details of the instrument
Indirect calorimetry	Sensormedics Vmax 29, Holland
Impedance analysis (Body Fat %)	TANITA, Model: TBF-105, TANITA UK Ltd., Made in USA
Blood pressure & resting HR	OMRON, Digital Blood Pressure Monitor OMRON Electronics Ltd, Model: HEM-405C
Body mass	John White & Son, (Weighing Machines) Ltd., Made in Scotland
Height	Holtain Ltd., Made in Britain

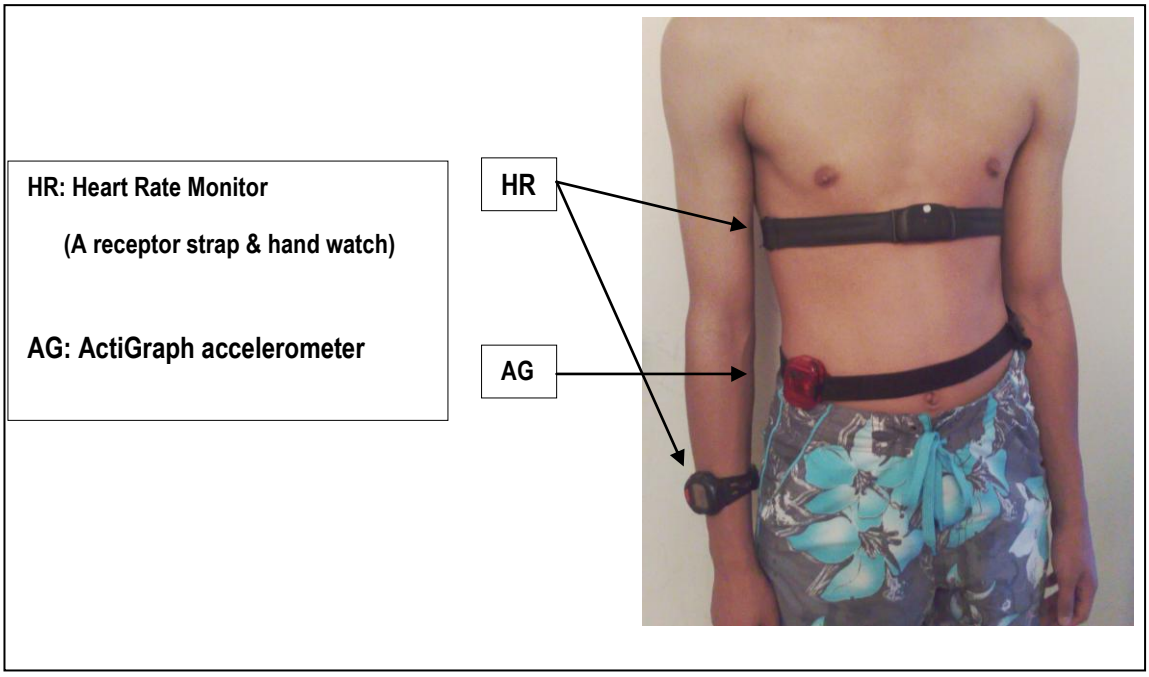


Figure 5.1 Illustration of the way of attaching ActiGraph device and HR monitor.

During the initial visit (first visit), both AG and HR were set up for the participant based on his/her characteristics and a code number was recorded in a record sheet. AG was set in counts per 15 second. Then participants were asked to wear the devices for two blocks of six days of physical activity monitoring. In each block of six days, participants were instructed to wear both devices as shown in Figure 5.1. Furthermore, they were asked to attach and remove both devices (AG and HR monitor) at the same time each day and to wear them for at least 10 hours of waking time (usually from morning until evening) as recommended by previous evidence (Harrell *et al.*, 2005; Reilly *et al.*, 2004).

In addition, they were provided with a physical activity log to record all physical activity of moderate intensity or more. The moderate, vigorous and very vigorous PA intensities were described with some examples. The PA log would be helpful if participants removed both devices whilst performing physical

activity with direct contact such as Judo or water activities such as swimming, as the devices are not waterproof. In the same log (PA log) they were asked to record the time they put the devices on or took them off. Each day, participants were asked to attach the AG device and remove it every measured day. Because the heart rate monitor needed to be started and stopped each time they wore it; each participant was provided with an instruction leaflet on how to use the HR monitor.

All instructions leaflets, PA log, information sheet as well as the two devices were provided to each participant in a plastic carrier file with his/her code number. All participants were provided with contact details of the principal researcher so they were able to contact him if they had any problems. At the end of each six days, each participant was asked to attend to the lab and return the plastic files including the devices as well as the logs. Data of AG and HR were downloaded to the computer and saved in MS Excel files. Then both the AG and HR monitor prepared for the next block of 6 days and the same procedures of retrieve and store data from both devices were taken.

5.2.2.3 Data analysis and statistics

Data obtained from AG were processed and analysed by a custom-written programme MAHUffe.exe available from (www.mrc-epid.cam.ac.uk). (Appendix D)

Activity data were cleared from periods when AG was not worn by excluding consecutive strings of zeros lasting 20 minutes or more (Harrell *et al.*, 2005). All

days of less than 10 hours of recorded data were excluded (Harrell *et al.*, 2005; Reilly *et al.*, 2004). Participants who had less than 3 days of acceptable data were excluded from the group (Reilly *et al.*, 2004). The main variable from AG was the average intensity of physical activity (mean and standard deviation of counts.min^{-1}). Puyau's cut-points for assessing habitual physical activity for children and adolescents were used for classifying PA into sedentary (<800 counts.min^{-1}), light (800-3200 counts.min^{-1}), moderate (3200-8200 counts.min-1), and vigorous (>8200 counts.min^{-1}) (Puyau *et al.*, 2002). These cut-points were developed by Puyau and colleagues using AG model MTI 7164. Although the output of the new AG model (GT1M) used in the present study has been found to be lower by 9% than MTI 7164, both models have been also found to be comparable in assessing moderate and vigorous activity intensity in children when the same epoch is used (Corder et al. 2007).

While there is a controversy regarding which cut-points to use (Reilly *et al.*, 2004; Ekelund *et al.*, 2004), the Puyau's cut-points were validated against energy expenditure during directly monitored free-living physical activity in children and adolescents (6-16yr) using whole body calorimetry (Puyau *et al.*, 2002), which is more likely to provide valid assessment of physical activity intensity versus other cut-points which have been developed based on laboratory tests such as Trost's cut-points (Trost *et al.*, 2002). The amount of time (minute) spent in each level of PA was calculated as well as the total registered time using the MAHUffe software programme.

In addition, habitual physical activity was also assessed using HR monitoring (Polar S-625X^{TM}). Heart rate data from each individual participant was

combined with his/her METs values that were obtained from the measured VO_2 during the laboratory test conducted walking on a treadmill in different speeds. They did this exercise test to determine their individual HR to VO_2relationship. The test started with setting up the AG and HR monitor for the participant. Then participant was asked to setting on a chair in a rest and comfortable position (the chair was on the treadmill). Mouth piece and nose clip were put comfortably and procedures of the tests were explained to the participant.

Following five minutes for resting VO_2 and HR measurement, the participant was asked to stand up and the chair was taken off the treadmill and the exercise was started with walking on the treadmill in different speeds ($3km.h^{-1}$, $4.5km.h^{-1}$ and $6km.h^{-1}$) as light and moderate and somewhat vigorous intensities. The duration was 5 minutes for each speed level. When the test was completed, data including VO_2 and HR were stored in MS Excel files for further analysis. The assessment of physical activity levels was calculated for each participant using the equation of the linear regression between METs and HR. the METs were calculated based on the measured VO_2 for each individual. Because children and adolescents have relatively higher resting VO_2 than adult's ($3.5\ ml.kg^{-1}.min^{-1}$), the resting VO_2 for each age and sex group were calculated (the resting VO_2 which equivalent to 1MET for 12-17yr adolescents = $4.15\ ml.kg^{-1}.min^{-1}$) and this is very close to the previously published values for children and adolescents (Harrell *et al.*, 2005). Linear regression equation between METs and HR was applied on the measured HR in free-living activity for each participant. Habitual physical activity for each participant was classified

based on HR that was equivalent to light <3 METs, Moderate ≥ 3–6 METs, vigorous > 6–9 METs and very vigorous > 9 METs.

Furthermore, new cut-points were developed for each individual participant in the current study. These cut-points were created by calculating the measured energy expenditure using indirect calorimetry and the counts per minutes that were obtained from AG during the laboratory walking test as described above. The developed cut-points in the current study were modified based on the children and adolescents equivalent of METs that were obtained from the lab test.

Statistical analyses were conducted using SPSS software version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). At least an alpha level of 0.05 was used for all analyses to indicate statistical significance. All data are summarised as means ± standard deviation (±SD) for the absolute and the percentage values. Independent-samples T-tests were used to compare the measured values. Moreover, One-sample T-test was used to compare the data from the present study with the mean values obtained in other studies on adolescents.

5.3 Results

A total of thirty two apparently healthy adolescents (males n=16, females n=16) completed their participation in the present study and provided valid measurements that met all inclusion criteria.

5.3.1 Habitual physical activity in adolescents assessed by Actigraph.

The results of the habitual physical activity obtained from AG for all participants and for each gender separately are summarised in Table 5.3.

Although there were no significant differences between males and females in number of days and registered time, males showed significantly higher mean counts per minute per day and active time as well as higher moderate and MVPA values than female adolescents (*P*=0.02, 0.01,0.01 and 0.01 respectively).

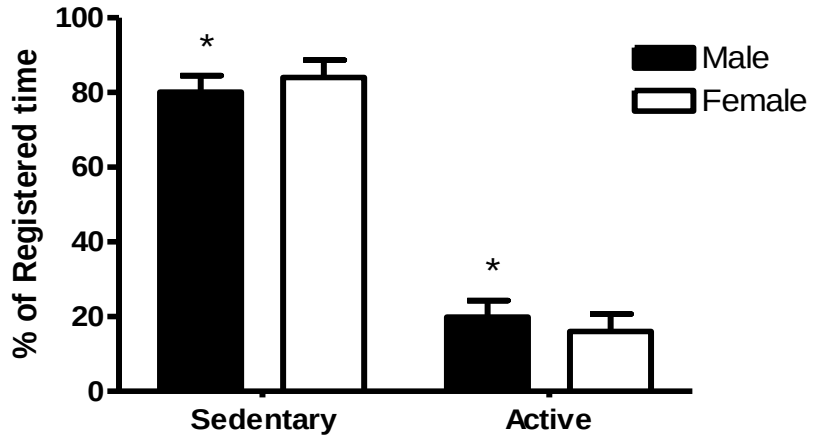
Table 5.3 Mean (±SD) of physical activity data for 32 adolescents (Male n=16, Female n=16) using Puyau's cut-points *et al.* (2002).

PA Parameters	All Adolescents	Males	Females
Number of Days	8.2 ±2.7	8.4 ±2.4	8.0 ±3.0
Registered Time (Min.day^{-1})	747.4 ±59.5	761.9 ±64.3	733.0 ±52.2
Counts.min^{-1}.day^{-1}	533.5 ±168.2	600.8 ±149.6	466.2 ±162.6 *
Sedentary (Min.day^{-1})	613.7 ±63.3	611.4 ±70.6	616.0 ±57.3
Active (Min.day^{-1})	133.8±36.2	150.5 ±29.5	117.0 ±35.1 **
Light (Min.day^{-1})	104.5 ±36.2	113.7 ±34.0	95.3 ±26.5
Moderate (Min.day^{-1})	26.1 ±14.6	33.1 ±13.0	19.0 ±12.8 **
Moderate to Vigorous (MVPA) (Min.day^{-1})	27.5 ±15.7	34.9 ±14.6	20.2 ±13.5 **
Vig. & Very Vigorous (V&Vvig.) (Min.day^{-1})	3.2 ±4.8	3.5 ±5.5	2.7 ±4.1

* Significant different from males at *P*<0.05

** Significant different from males at *P*<0.01

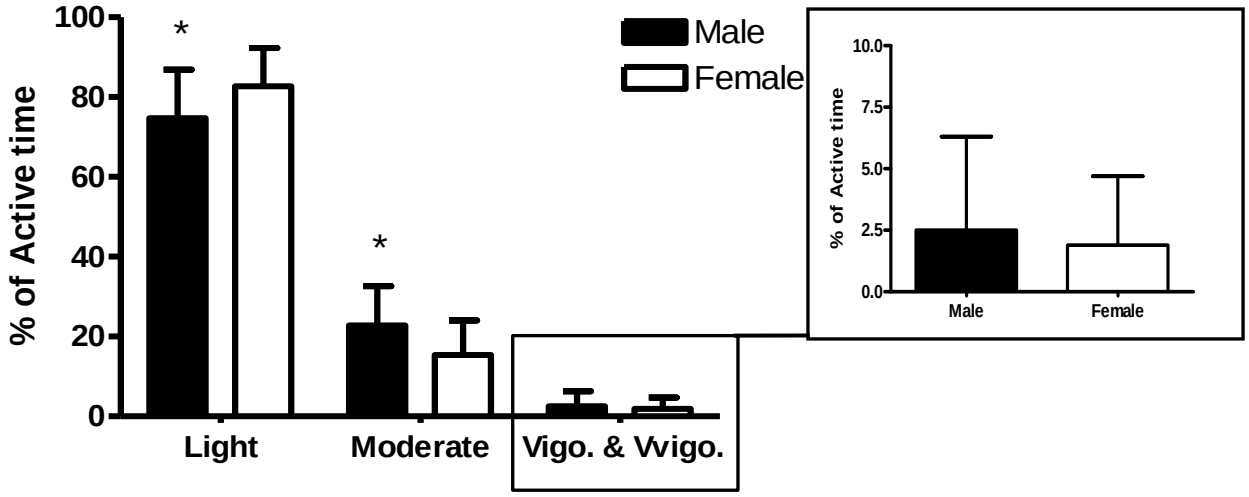
Data was analysed and presented as percentage values in order to normalize variables. Therefore, percentage of registered time spent in sedentary and activity time was compared between genders. Data in Figure 5.2 showed that females have significantly higher percentage of registered time spent in sedentary behaviour than males, and a lower percentage of registered time spent in total activity time than males (both at *P*=0.02).



* Significant different from males at *P*<0.05

Figure 5.2 Mean(SD) of percentage of registered time spent in sedentary and activity (Male=16, Female=16) adolescents.

On the other hand Figure 5.3 indicates that percentage time spent in each physical activity levels showed that males were significantly lower than females in light activity but higher in moderate activity. There were no differences between groups in vigorous and very vigorous activity levels.



* Significant different from males at *P*<0.05

Figure 5.3 Mean(SD) of percentage of active time spent in light, moderate and vigorous & very vigorous (Male=16, Female=16) adolescents.

5.3.2 Habitual physical activity in adolescents assessed by HR monitor.

Habitual physical activity of the 32 adolescents participants were assessed using HR monitor combined with individually assessed HR to METs relationship. The result of physical activity status is summarised in Table 5.4.

The results indicated that there were no differences between male versus female participants except in the vigorous and very vigorous level (*P*=0.04).

Table 5.4 Mean (±SD) physical activity data of the 32 adolescents using HR monitor (Males n=16 and Females n=16).

PA Parameters	All Adolescents	Males	Females
Number of Days	7.6 ±2.3	8.2 ±2.1	6.9 ±2.2
Registered Time (Min.day^{-1})	661.6 ±38.6	674.6 ±31.8	648.5 ±40.4
Sedentary (Min.day^{-1})	325.7 ±89.1	320.9 ±90.6	330.5 ±87.1
Active (Min.day^{-1})	335.8 ±96.5	353.7 ±97.6	318.0 ±91.6
Light (Min.day^{-1})	239.2 ±84.4	244.6 ±85.8	233.8 ±82.5
Moderate (Min.day^{-1})	84.1 ±35.2	93.2 ±29.0	75.0 ±38.6
Moderate to Vigorous (MVPA) (Min.day^{-1})	93.3 ±38.6	104.9 ±32.4	81.8 ±40.9
Vig. & Very Vigorous (V&Vvig.) (Min.day^{-1})	12.5 ±9.2	15.9 ±10.4	9.2 ±5.9*

* Significant different from males at $P<0.05$

The percentage of registered time spent in each physical activity level (Figure 5.4) indicated that there were no differences between males and females in percentage of registered time spent in sedentary or activity time.

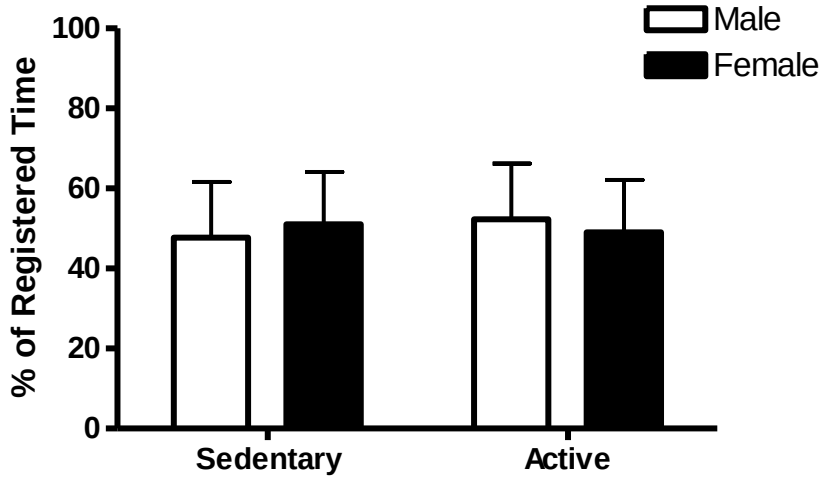


Figure 5.4 Mean(SD) of percentage of registered time spent sedentary and active time for adolescents (Male=16, Female=16) using HR defined thresholds.

Similarly, Figure 5.5 illustrated that the percentage of active time spent in light, moderate and vigorous & very vigorous. Although there were significant differences between males and females in vigorous & very vigorous level in the absolute time, percentage time spent in this level of activity was not significantly different between genders.

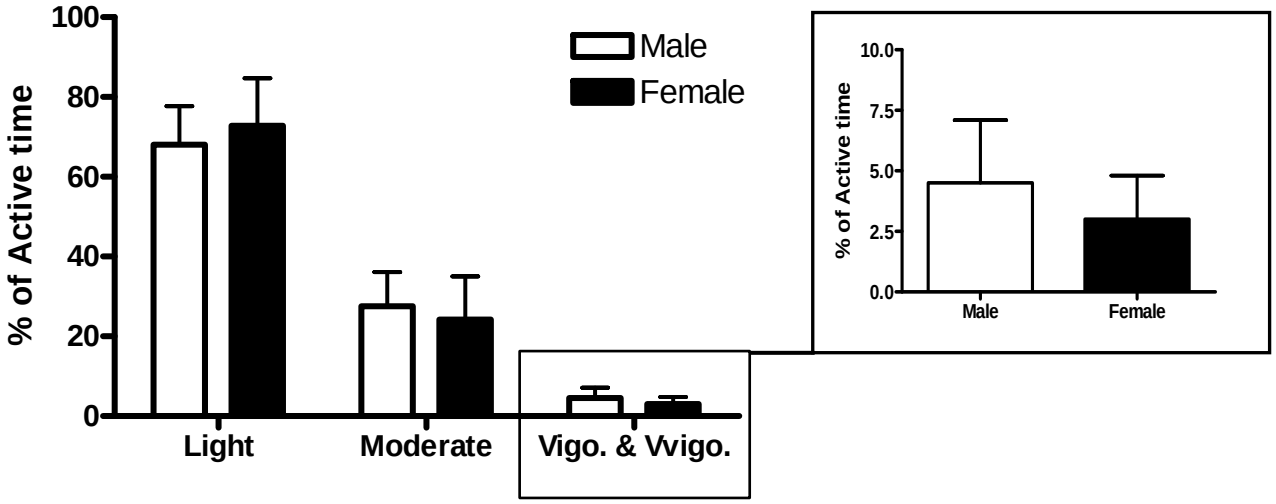


Figure 5.5 Mean(SD) of percentage of active time spent in light, moderate and vigorous & very vigorous for adolescents (Male=16, Female=16) using HR defined thresholds.

5.3.3 Comparison to other data on habitual physical activity of adolescents.

The results from the accelerometry in the present study were compared to the data of physical activity assessed by accelerometry (Actigraph) in two similar studies. The number of participants, age and accelerometers used in each study are presented in Table 5.5. In the first study Riddoch and colleagues (2004), assessed the habitual physical activity of 2185 adolescents (9 and 15yr) including males and females in four European countries (Denmark, Portugal, Estonia and Norway) using the MTI Actigraph model 7164 (Riddoch *et al.*, 2004). In the present study, only PA data of the 15yr participants in Riddoch's study will be used. They selected 1500 counts per minute as a MVPA threshold for the 15yr group. The selected cut-points were calculated using the developed Freedson's equation which is developed based on lab exercise using walking and jogging on a treadmill (Freedson *et al.*, 1997). Riddoch *et al.* (2004) found

that 1706 counts represented an energy expenditure of 3 METs for their 15yr participants and they selected the nearest value of 1500 counts per minutes as a MVPA threshold for 15yr olds, but they did not provide any further justification rather than the equation has been used in a previous study (Trost *et al.*, 2002).

Table 5.5 Number of participants, ages, and accelerometer types used in the present study, Riddoch's study and in Jago's study.

	The present study		**Riddoch *et al.* study** [1]		**Jago *et al.* study** [2]	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Age	14.6 +1.8	14.0 +1.5	15.5 +0.5	15.4 +0.5	13.3 +0.5	13.3 +0.5
No. of participants	16	16	Total of 2185		37	44
Accelerometer type	Actigraph GM1T		MTI Actigraph Mod. 7164		MTI Actigraph Mod. 7164	

1. Riddoch CJ. *et al.* 2004.

2. Jago R. *et al.* 2005.

The second study by Jago and colleagues (2005) used the MTI Actigraph model 7164. They used Puyau's cut-points which were also chosen for the present study. They studied 81 American adolescents (age 13.3 $\pm$.05yr, including males n=37 and females n=44) (Jago *et al.*, 2005). Physical activity data from both studies (Riddoch's and Jago's) were compared to the present study and the results are summarised in Table 5.6. The results reveal that males spent more time engaged in MVPA than females in all studies. On the other hand, the results of the American adolescents study (Jago *et al.* 2005) showed the lowest values in light and MVPA levels than the adolescents in the present study.

Table 5.6 Comparison between the physical activity data of the present study to two similar studies (one in American adolescents and one in 4 European countries adolescents)

Physical activity parameters	The present study		Riddoch *et al.* study[1]		Jago *et al.* study[2]	
	Males	Females	Males	Females	Males	Females
No. Days	8.4 ±2.4	8.0 ±3.0	3.7	3.7	4 ⊤	4 ⊤
Reg. T (min.day^{-1})	761.9 ±64.3	733.0 ±52.2	823.8 +212.4	810.3 ±211.1	1080 ⊤	1080 ⊤
Counts.min^{-1}.day^{-1}	600.8 ±149.6	466.2 ±162.6*	615 ±228	491 ±163*	NA	NA
Sed. (min.day^{-1})	611.4 ±70.6	616.0 ±57.3	NA	NA	924.5 ±98.6 ⊤	985.8 ±81.6 * ⊤
Sed. (% of Reg. T)	80.1 ±4.4	84.4 ±4.7*	NA	NA	85.6 ±9.2	91.3 ±7.6
Light (min.day^{-1})	113.7 ±34.0	95.3 ±26.5	NA	NA	128.1 ±13.4	184.3 ±13.3 * ⊤
Light (% of active time)	75.5 ±12.2	81.5 ±9.6*	NA	NA	82.3 ±6.9	95.0 ±6.7*
MVPA (min.day^{-1})	34.9 ±14.6	20.2 ±13.5**	99 ±45	73 ±32*	27.5 ±6.8	9.8 ±3.1 ** ⊤
MVPA (%of active time)	23.2 ±9.8	17.3 ±8.6*	NA	NA	17.6 ±3.5	5.0 ±1.5**
Days.wk^{-1} of MVPA≥60min	4.1	2.4	7	7	3.2	1.1
Participants met Guidelines (%)[a]	25.0	12.5*	81.9	62.0*	NA	NA

* Significant difference from male at $P<0.05$, ** Significant difference from male at $P<0.01$

⊤ Significant difference from the same gender in the present study at $P<0.01$

⊤ Significant difference from the same gender in the present study at $P<0.000$

[a] 1hour of MVPA in ≥5d.wk^{-1}., 1. Riddoch CJ. *et al.* 2004., 2. Jago R. *et al.* 2005

In the European study (Riddoch *et al.*, 2004), the data of Riddoch and colleagues appears to indicate that both genders of the adolescents spent more time in MVPA than the adolescents in the present study. However, the mean counts per minutes per day were not significantly different from the mean counts per minutes per day in the present study (*P*=0.17).

The present study was a cross-sectional study, therefore, it was decided that the data of the present study may need to be reanalysed using the same cut-points that were used in Riddoch's study. Despite the validity of the cut-points that have been used in Riddoch's study, using these cut-points may help to make the comparison between data of the adolescents in both studies based on the same cut-points. Table 5.7 shows that there was no significant difference between the present study versus Riddoch's study in counts per minutes per day (male *P*=0.99, female *P*=0.87) or in MVPA (male *P*=0.09, female *P*=0.32). Regardless of how active the adolescents are, adolescents in both studies seem to engage in similar habitual physical activity.

Table 5.7 Comparison of physical activity data from the present study versus Riddoch's study using the same cut-points.

Physical activity parameters	The present study using Riddoch's cut-points		Riddoch *et al.* study[1]	
	Males	Females	Males	Females
No. Days	**7.9 ±2.6**	**7.7 ±3.2**	**3.7***	**3.7***
Reg. T (min.day^{-1})	**753.8 ±65.9***	**742.2 ±39.0***	**823.8 +212.4***	**810.3 ±211.1***
Counts.min^{-1}.day^{-1}	**612.3 ±165.8**	**498.4 ±182.4**	**615 ±228**	**491 ±163**
MVPA (min.day^{-1})	**89.7 ±20.4**	**66.9 ±23.8**	**99 ±45**	**73 ±32**
Average days/wk ≥60min of MVPA	**7**	**7**	**7**	**7**
Participants met Guidelines (%)[a]	**100.0**	**68.8**	**81.9**	**62.0**

* Significant difference from the same gender in the present study at $P \leq 0.001$

[a] 1hour of MVPA in ≥5days/wk.

1. Riddoch CJ. *et al.* 2004.

Habitual physical activity has been assessed in Australian adolescents by Hardy and colleagues (2008). They compared physical activity status for adolescents in New South Wales (Australia) between 1997 and 2004 using the New South Wales Schools Fitness and Physical Activity Survey (NSW SFPAS, 1997). Only physical activity data of the participants in 2004 was compared with the similar data in the present study. Table 5.8 presents PA data of the Australian adolescents in 2004 and showed that at least two thirds of the participants were active and met the recommended guidelines of ≥ 60 min.d^{-1} regardless of age and genders. Males appeared to be more active than females and the differences between genders reduced as age increased.

Table 5.8 Physical activity data for Australian adolescents (2004).

	Males		Females	
Age (yr)	**13.3 ±0.4**	**15.3 ±0.4**	**13.3 ±0.4**	**15.3 ±0.4**
Participants (*N*)	**408**	**555**	**393**	**415**
MVPA (min.d^{-1})§	**112.9 [75.7, 172.9]**	**105.0 [61.4, 160.4]**	**90.0 [59.3, 134.3]**	**70.4 [41.4, 123.6]**
Prevalence (%) of ≥60min.d^{-1}	**87.3**	**77.9**	**76.8**	**59.8**
Measuring method	**The NSW Schools Fitness and Physical Activity Survey (SFPAS, 1997)**			

§ Median [interquartile range (IQR)]

The physical activity levels of the adolescents in the present study were also assessed using cut-points developed from the adolescents' measured VO_2 during the laboratory test which included walking on a treadmill at different speeds. The results are summarised in the Table 5.9. Similar to the results found in Puyau's cut-points, there were significant differences between genders in active time, counts.min^{-1}, moderate and MVPA time (P=0.03, 0.03, 0.01 and 0.01 respectively). All participants were shown to be active and most of them met the recommended guidelines for health and wellness.

Table 5.9 Mean (SD) of physical activity data from the present study using created cut-points for 32 adolescents (Males n=16 and Females n=16).

Physical activity parameters	**The present study using created cut-points**	
	Males	**Females**
No. Days	8.6 ±2.3	7.9 ±3.3
Reg. T (min.day^{-1})	756.4 ±70.4	741.4 +38.8
Counts.min^{-1}.day^{-1}	609.5 ±164.9	463.5 ±162.6 *
Sedentary (Min.day^{-1})	593.3 ±74.4	610.9 ±51.4
Active (Min.day^{-1})	163.1 ±35.3	130.5 ±36.4 *
Light (Min.day^{-1})	83.6 ±26.5	75.2±20.4
Moderate (Min.day^{-1})	74.2 ±19.6	51.8 ±18.6 **
MVPA (min.day^{-1})	78.8 ±121.0	54.1 ±20.2 **
Vig. & Very Vigorous (V&Vvig.) (Min.day^{-1})	5.2 ±7.7	3.4 ±5.0
Average days/wk ≥60min of MVPA	7	6.3
Subjects met Guidelines (%)[a]	100	73.3
Resting VO_2 (ml.kg.min^{-1})	4.15 ±0.78	
Counts.min^{-1} at 1 MET § (at rest)	691	
Counts.min^{-1} at 1.6 – 2.9 METs (Light)	1107–1858	
Counts.min^{-1} at 3 – 5.9 METs (Moderate)	1859 – 6695	
Counts.min^{-1} at ≥6 METs (Vigor. & Vvgor.)	≥6696	

* Significant different from males at *P*<0.05

** Significant different from males at P<0.01

a >1hour of MVPA in >5days/wk.

§ 1MET = 4.15 ml.kg.min-1

5.4 Discussion:

5.4.1 Habitual physical activity in adolescents assessed by Actigraph and HR monitor.

One of the primary aims of the present study was to assess the habitual physical activity for Scottish adolescents using objective measures (Actigraph GM1T and HR monitor). The results were dependent upon the instrument used (accelerometer or HR monitor) and the interpretation of the output data (applied cut-points). Actigraph has been developed and widely used in research as a valid and reliable accelerometer for children and adolescents (Puyau *et al.*, 2002; Freedson *et al.*, 1997).

In the current study, a new generation of Actigraph GTM1 (AG) was used, and physical activity intensities were assessed using Puyau's cut-points which were developed for children and adolescents based on free-living activities (Puyau *et al.*, 2002). The results obtained from AG showed that males were more active than females. Although the majority of participants were apparently healthy lean, both genders failed to achieve the recommended guidelines for adolescents (MVPA $\geq$1hr on $\geq$5d.wk^{-1}) for the purpose of health and wellbeing published by the World Health Organization in Europe (Currie *et al.*, 2004). On the other hand, different results were found when the habitual physical activity of the adolescents was assessed using HR monitors based on METs from the laboratory exercise walking test. HR method showed higher values comparing to AG method as HR data indicated that all participants met the recommended guidelines including vigorous and very vigorous intensity levels. The differences

in the results obtained from these two methods (AG and HR) are similar to the differences observed in adults study (chapter 3). Therefore, at least one of these two methods was not able to provide acceptable findings. Similar to what was observed in the adult study (chapter 3), the differences in the findings between AG and HR methods was probably due to the fact that the HR method was based on a limited range of physical activity intensities (only the laboratory exercise test (walking at 3, 4.5 & 6km.h^{-1})) which, in turn, provided higher values of time spent in each physical activity level. Moreover, the higher activity values obtained from the HR method were probably due to some physiological or psychological factors such as emotion and diet status which may affect the heart rate. Although, both methods have limitations, the AG method is more trustable as the AG method has been developed to assess free-living physical activity in children and adolescents (6-16yrs) and provides consistent output (Puyau *et al.* 2002, Reilly *et al.* 2004). Thus, AG with a proper method is recommended and more favourable for further studies.

One explanation is that AG is a locomotor device and the HR monitor is a physiological based assessment. Thus, HR is more likely to be affected by static physical activity as well as the emotional behaviour whereas AG responds only to the dynamic activity (Johansson *et al.*, 2006). The differences may not be noticeable when they both are used in a short term of physical activity behaviours such as during a football game but it may be more obvious in long term period such as monitoring physical activity over a week which include different factors that may influence HR such as emotional behaviours.

In comparison to AG using Puyau's cut-points, the HR monitoring method may not be as accurate as accelerometry as HR may overestimate physical activity assessment in children which has been observed previously (Emons *et al.*, 1992). This is due to the fact that HR usually is affected by physiological and psychological factors. For example, having a main meal (rich of protein and fat) may lead to increases in HR above normal resting rate. Moreover, adolescents living in a stage where there are many changes in their life conditions such as family problems which may affect their heart rate response especially in the psychological and emotional aspects. On the other hand, AG may not record the physical activity intensity during cycling or upper limbs activities such as arms as well as it does for activity using the lower limbs (Trost *et al.*, 2005). As observed in adults (Chapter 3), the trend of the results obtained from AG versus HR reading was observed in adolescents in the present study. In both adults and adolescents the AG produced lower values for physical activity compared to HR monitoring. However, the lower values of physical activity levels obtained from AG are partly due to the cut-points used in the current study. The cut-points developed by Puyau *et al.* (2002) for children and adolescents are higher compared to the other developed cut-points (Freedson *et al.*, 1997; Treuth *et al.*, 2004). For instance, Treuth's cut-points for MVPA is $\geq$3000 counts.min^{-1}, whereas Puyau's cut-point is $\geq$3200 counts.min^{-1}. In general, both measurement devices (AG and HR monitor) in the present study indicated that males were more active significantly than females in MVPA and in vigorous and very vigorous physical activity. However, for comparison of the activity behaviours between studies, the accelerometry cut-points and other related data interpretations used are clearly crucial in the conclusions that are made.

5.4.2 Comparison to data on habitual physical activity of adolescents from other studies.

The second primary aim of the present study was to compare the habitual physical activity of Scottish adolescents assessed by the accelerometry to physical activity data assessed by accelerometry from similar studies of adolescents from different countries. The results of the present cross-sectional study showed that Scottish adolescents engaged in similar physical activity levels found in other European adolescents (Riddoch *et al.*, 2004). Riddoch's study is the first study used objective measure (AG) for assessing habitual physical activity in a representative sample of European adolescents (Riddoch *et al.*, 2004). The cut-points they used were obtained from Freedson's published equation for assessing physical activity in children and adolescents (6-18yr) (Freedson *et al.*, 1997). They found that adolescents (15yr) of the four European countries (Denmark, Portugal, Estonia and Norway) were active enough to meet the recommended guidelines issued by the World Health Organization in Europe ($\geq$1hr of MVPA in $\geq$5days per week) for children and adolescents (Currie *et al.*, 2004).

Interestingly, despite differences between the present study and Riddoch's study in MVPA and percentage of participants meeting the recommended guideline, the average counts per minutes per day in Riddoch's study were not significantly different from the average counts per minutes per day in the present study. Therefore, to compare the present data with Riddoch's protocol all the analysis parameters used in Riddoch's study the cut-points (MVPA $\geq$1500counts.min^{-1}), 1min epoch and excluding zeros ($\geq$10minutes of zeros)

were applied to the data of the present study. The results showed that there were no significant differences between the adolescents in both studies in time spent in MVPA. Moreover, the same trend of results was found in both studies as males were found to be significantly more active than females.

However, the cut-points that have been used in Riddoch's study may not be as accurate as Puyau's cut-point. This is because the cut-points used in Riddoch's study were obtained from Freedson's equation that was developed based on a laboratory-test exercise on a treadmill (2walking & 1Jogging) for children (6-18yr) (Freedson *et al.*, 1997) and not based on a free-living activity as in Puyau's equation (Puyau *et al.*, 2002). The Freedson's equation provides relatively lower cut-points compared to Puyau's cut-points which, in turn, led to higher values for time spent in each of the physical activity intensity. Thus, Puyau's cut-points are more likely to be more accurate to assess the habitual physical activity for adolescents in free living activity simply because they were developed based on free-living activities (Puyau *et al.*, 2002). In Riddoch's study, they set the activity counts of the AG at 1min epochs which may not be a suitable epoch for children and adolescents as it may underestimate physical activities especially vigorous and very vigorous activity because such activity is rarely sustained for as long as 1minute. For these reasons a previous study has recommend that when AG is used for children and adolescents a low epoch such as 10s or 15s should be selected to estimate all physical activity intensities including vigorous and very vigorous (Trost *et al.*, 2005). Moreover, Riddoch *et al*. (2004) used $\geq$10minutes of consecutive zeros as a deletion data presuming that the participant was not attaching the device on the body. In turn,

the sedentary time may be less than the actual time because body movements of the adolescents are not the same as in young children and they may spend longer time periods reading or watching TV without movement.

When the same cut-points and method were used in both studies, the Scottish adolescents in the present study would appear to be similar to the other European adolescents in Riddoch's study in terms of gender effect on habitual physical activity as males were more active than females in both studies.

Data of habitual physical activity from American adolescents (13.3yr ±0.5) (Texas State) was used to be compared to the similar data in the present study (Jago *et al.*, 2005). Jago and colleagues (2005) assessed physical activity of American adolescents using the same accelerometry (AG), cut-points (Puyau's cut-points) and methods (excluded data ≥20mins of continues zeros) that have been used in the present study. The results of the comparison indicated that female adolescents of the current study spent significantly more time in MVPA than female adolescents in Jago's study whereas there was no significant difference between male adolescents in both studies for MVPA. However, adolescents in both studies were below the recommended guidelines of physical activity for children and adolescents (MVPA ≥1hr at least ≥5d.w^{-1}) (Currie *et al.*, 2004). In Jago's study, there were 15% of the adolescents were at risk of overweight (≥85th to <95th percentile) and 16% of them were overweight (≥95th percentile).

On the other hand, there were only 9.4% of the adolescents in the present study were overweight (≥95th percentile for age). Moreover, Jago and

colleagues did not mention which epoch they selected to assess physical activity. However, they mentioned that due to the extremely low levels of vigorous and very vigorous activity (<2min.d^{-1}) obtained from AG, they only reported MVPA instead. It is probably that they have used a 1min epoch. If the probability is correct, then the high intensity of physical activity (e. g vigorous and very vigorous) of the adolescents may be underestimated (Trost *et al.*, 2005). Overall, the results of the previous discussed studies including the present study showed that European adolescents including Scottish adolescents seem to be more active compared to American adolescents (Texas State). Moreover, male adolescents are more likely to spend more time in MVPA compared to female adolescents. Assessing physical activity using accelerometry with proper cut-points (Puyau's cut-points) and methods showed that all adolescents in the discussed studies including the current study were below the recommended physical activity guidelines for adolescents (Strong *et al.*, 2005).

Habitual physical activity data of the Scottish adolescents in the current study was compared to the habitual physical activity data of the Australian adolescents obtained from a survey. Hardy and colleagues (2008) assessed physical activity of the adolescents using the same survey that was used in 1997 for the purpose of comparing physical activity levels for the adolescents (13.3yr ±0.4, 15.3yr ±0.4) between 1997 and 2004 (Hardy *et al.*, 2008). Data from the Hardy's study indicated that Australian adolescents in both age groups (13.3yr, 15.3yr) were more active than Scottish adolescents in the present study (all at P=0.00). Although the subjective measure such as the survey is

inexpensive and it can be helpful for covering a large percentage of the researched population, it has some limitations that may affect the accuracy of the results. For example, MVPA assessment through the self-report has been found to be overestimated (Hardy *et al.*, 2008). Other studies demonstrated that self-report may affect the accuracy of physical activity assessment in children and adolescents in terms of frequency and duration (Kohl *et al.*, 2000).

Habitual physical activity was also assessed in the present study using cut-points created for each individual from the laboratory walking test. The results showed the same trend as males spent significantly more time in moderate and MVPA than females (both P=0.01), but both genders met the recommended guideline of physical activity (Strong *et al.*, 2005). Supporting the previous suggestion, the created cut-points in the present study seem to be inappropriate to assess the habitual physical activity in adolescents because the created cut–points were developed based on a lab test and not from lifestyle activity (Welk, 2005). Thus, the result was relatively higher compared to the results obtained from Puyau's cut-points.

It has been mentioned previously that assessing physical activity for children and adolescents accurately is difficult (Armstrong and Welsman, 2006; Bringolf-Isler *et al.*, 2009). Thus, more research is needed in order to improve the available objective instruments so that physical activity can be accurately assessed especially in children and adolescents. It would seem that further work on achieving suitable cut-points is paramount to obtain a picture of the activity status of the adolescent populations in any country.

The present study was well controlled in terms of procedures and design. However, the results of the present study would be more conclusive if the sample size was equal to or more than the required number for each group (> 28 participants in each group (male vs. female)). Due to the time limit given to complete the current study, the sample size of the current study was below the number required to generalise the results. Therefore the results of the present study provided useful preliminary knowledge about the habitual physical activity of the adolescents living in central Scotland. A longitudinal study or a study with relevant interventions would be more confident to generalise the findings about physical activity status of adolescents in Scotland, and could provide useful information to the Scottish strategy of “make Scotland more active”.

5.5 Conclusion

Habitual physical activity has been assessed in adolescents in different countries around the world using different data-collection methods and instruments (Reilly *et al.*, 2004; Ekelund *et al.*, 2004; Livingstone, 2001; Kohl *et al.*, 2000). However, the prevalence of obesity among children and adolescents has increased over the last decades in different countries around the world including Europe, North America, and Australia (Jago *et al.*, 2005; Currie *et al.*, 2004; Livingstone, 2001; Hardy *et al.*, 2008; Schneider, 2000).

The question is; are the examined adolescents active enough to improve health and wellbeing? If the answer in some cases is yes, then why is the prevalence of obesity and overweight among adolescents still increasing? This is, partly, what Riddoch and colleagues mentioned that in terms of public health policy, as

it may now be necessary to question whether the current activity recommendations for children and adolescents are appropriate. Further analyses -assessing dose- response relationships between activity and health risk factors – may provide more clinically based activity recommendations or in other words, maybe the Puyau's cut-points are correct. However if the answer in other cases is no, then standardising the definition of physical activity for adolescents, data collection methods, and instruments used is important in order to assess the global trends of the habitual physical activity in adolescents (Hardy *et al.*, 2008). Consequently, further studies may be needed in order to investigate the relationship between the habitual physical activity and health status in terms of cardiovascular and metabolic diseases risk markers for adolescents.

Chapter 6: The association between physical activity status and cardiovascular and metabolic disease risk markers in Scottish adolescents

6.1 Introduction

Physical activity in early age such as adolescence may help to promote a healthy lifestyle in adulthood and may reduce chronic diseases such as cardiovascular and metabolic diseases (Davis, 1980; Hallal *et al.*, 2006; Martins *et al.*, 2010). Moreover, a number of chronic diseases such as heart disease, osteoporosis and some other diseases that appear in adulthood, in fact, are developed in childhood and during adolescence (Parsons *et al.*, 1999). Therefore, an adequate improvement in physical activity must start at earlier age such as childhood (Hallal *et al.*, 2006; Currie *et al.*, 2004).

The effect of physical activity on the key risk markers of cardiovascular and metabolic diseases in adolescents has been investigated in a number of studies (Hulver *et al.*, 2003; Kelly *et al.*, 2007; Blüher *et al.*, 2006; Ischlander *et al.*, 2007; Knox *et al.*, 2009; Rizzo *et al.*, 2008; Rubin *et al.*, 2008; Isasi *et al.*, 2003). However, the effect of physical activity status per se on the cardiovascular and metabolic disease risk markers is not fully clear because the results of previous studies have not fully agreed about the effect of physical activity on the cardiovascular and metabolic risk markers in adolescents. In previous studies, Hulver *et al.* (2003) and Kelly *et al.* (2007) inferred the influence of the physical activity intervention on the examined cytokines to the reduction in adiposity (e.g. in the absence of weight loss, exercise training alone

does not improve the adipokine profile including adiponectin, IL-6 TNF-α and CRP). In contrast, Bluher *et al.* (2006) and Ischlander *et al.* (2007) demonstrated that the effect of physical activity, or exercise training, on some of the cytokines were positive and independent of the change in body fat.

Most of the previous studies have investigated the influence of body fat and exercise on cardiovascular and metabolic disease risk markers (Hulver *et al.*, 2003; Kelly *et al.*, 2007; Blüher *et al.*, 2006; Ischlander *et al.*, 2007). There are some studies which have investigated the effect of physical activity on these blood markers in lean and apparently healthy adolescents. However, most of them have used subjective physical activity measures such as a questionnaire (Ischlander *et al.*, 2007; Rubin *et al.*, 2008). Few studies have examined the association between physical activity and clustered metabolic risk using objective measures (Steele *et al.*, 2008). More investigations are needed to examine the effect of the physical activity status on the key risk markers of cardiovascular and metabolic disease including the traditional blood risk markers such as blood lipids and fatty acids (omega 3 and omega 6) and more recent markers such as adiponectin, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and high sensitivity C-reactive protein (hsCRP) alongside use of objective measures such as accelerometry and heart rate monitoring (HR). In the previous chapter (chapter 5), habitual physical activity of adolescents was assessed using an objective measure and the most appropriate cut-points were applied on the raw data which were developed based on free-living activity for children and adolescents (Puyau *et al.*, 2002). The results were undesirable because only a few of the participants met the recommended guidelines for

physical activity for children and adolescents especially in females (25% of male and 12.5% of female). Thus, the contribution of the current study is to further explore the relationship between physical activity and cardiovascular and metabolic disease risk markers in order to improve public health promotion. More evidence may be needed to help in convincing policy-makers to take forward steps towards encouraging young people to spend more time in sufficient physical activity for health and disease prevention (Let's make Scotland more active: A Strategy for physical activity, 2003). Following on from the habitual physical activity assessment using objective measures in adolescents, the question is whether the physical activity status of the adolescents is potentially detrimental to their future health and wellness? Thus, the primary aim of the present study was to investigate some of the important cardiovascular and metabolic disease risk markers in relation to physical activity status in apparently healthy lean adolescents, and secondarily, to compare the results to similar studies in lean adolescents.

6.2 Materials and Methods

6.2.1 Subjects

In the present study, only 21 of the 32 participants in the previous chapter gave consent to return to the laboratory to obtain a morning fasted blood sample (males n=13 and females n=8). One of the boys' samples was excluded because his blood results were unusual and it appears he may have eaten something before the blood test (high fasting insulin of 65.8 µIU/ml).

Therefore, the total samples are 20 obtained from the 20 lean adolescents (males n=12 and females n=8). Participants were classified as lean when their BMI <85th centile for age and as obese when their BMI ≥98th centile for age.

The power calculation for the sample size required at least 20 participants in each group (male vs. female). Thus, the present study could be considered as a pilot study and as first step for further studies.

Table 6.1 illustrates the physical characteristics of the participants. Significant differences between males versus females were found in height, BMI, percentage of body fat and resting heart rate (HR).

Table 6.1 Physical characteristics presented as means ±SD for 20 adolescents (12-17yr), (males n= 12, females n= 8).

Physical characteristics	**All adolescents**	**Male**	**Female**
Age (yrs)	14.8 ±1.7	14.9 ±1.8	14.5 ±1.5
Mass (Kg)	56.5 ±9.2	56.3 ±9.3	54.4 ±13.3
Height (cm)	168.4 ±10.3	170.6 ±12.1	161.4 ±34.8*
BMI (Kg.m-2)	20.2 ±2.9	19.1 ±1.4	21.8 ±4.0*
Percentile for age	57th ±30th	49th ±26th	69th ±30th
Body Fat%	17.6 ±8.3	12.0 ±4.7	26.0 ±4.4**
Fat Free Mass (Kg)	46.6 ±9.0	49.8 ±9.7	41.9 ±5.2
Mean arterial pressure (mmHg)	82.3 ±7.6	84.8 ±6.3	78.6 ±8.2
Resting heart rate (bpm)	63.7 ±11.8	59.3 ±11.0	70.1 ±10.5*
Predicted VO_{2max} (L.min^{-1})	3.0 ±0.9	3.2 ±1.0	2.9 ±1.0

* Significantly different from male group at P <0.05

** Significantly different from male group at P <0.001

Then body mass index (BMI) (body mass(Kg) x height(m^2) was calculated using a BMI reference chart for UK children (UK90) (Cole *et al.*, 1995). Participants were asked to attend early morning (7- 9am) after an overnight fast to the laboratory on one occasion. A total blood sample of 10ml was collected by a trained person from a peripheral vein. The habitual physical activities and physical measurements of all participants were assessed by objective instruments as described in Chapter 5. All blood samples were collected into vacutainer tubes containing K_3EDTA by a trained repetition person from an antecubital vein with the person in a seated position. The blood sample was centrifuged at 4^oC for 10 minutes to isolate plasma. The plasma was stored in each of 5 aliquots for subsequent analyses:

- 1ml for fasting triglycerides, total cholesterol and fatty acid analysis.
- 0.5ml for fasting glucose.
- 1ml for leptin and adiponectin.
- 1ml for tumor necrosis factor-alpha (TNF-α), high sensitivityC-reactive protein (hsCRP) and interleukin-6 (IL-6).
- 0.5ml for insulin.

6.2.2 Assay Procedures:

The Enzyme-Linked Immunosorbant Assay (ELISA) assay procedures were applied to leptin, adiponectin, TNF-α, hsCRP, IL-6, and insulin in the present study. The Enzymatic Assay procedures were applied to triglyceride, total cholesterol, free fatty acid and glucose. Lipid profile ratio of arachidonic acid to

eicosapentaenoic acid (ARA/EPA), [also known as (20:4n-6)/(20:5n3)] and the ratio of % n-3 to the total highly unsaturated fatty acid (HUFA) were assayed using the Ideal Omega Test provided on online (Ideal Omega test (no publication date). Available: http://www.idealomegatest.com). The procedure of insulin ELISA will be described in detail as an example for the leptin, adiponectin, TNF-α, hsCRP, IL-6, and insulin. Glucose oxidase assay procedure will be described as an example of the enzymatic assay of triglyceride, total cholesterol, free fatty acid and glucose. Omega 3 (GC-MS) assay will be described as an example of Omega 3 and Omega6.

ELISA Insulin assay:

Assay methods for ELISA and enzymatic methods were as described in previously in chapter 4.

Physical activity assessment

Habitual physical activity was assessed using an Actigraph accelerometer and Puyau's cut-points were applied for assessing habitual physical activity for children and adolescents. The Puyau's cut-points were developed based on free-living physical activity for children and adolescents (6-16yr). The cut-points classify the physical activity intensities as light <3METs, moderate ≥3–6METs, vigorous >6–9METs and very vigorous >9METs (Puyau *et al.*, 2002).

6.2.3 Data analysis and Statistics

Data was sorted and stored in different excel worksheets. All measured variables were used to compare low active group versus active group.

Statistical analyses were conducted using SPSS software version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). At least an alpha level of 0.05 was used for all analyses to indicate statistical significance. Data are summarised as mean and standard deviation ($\pm$SD). Independent-samples t-tests were used to compare the measured values for low active versus normally active groups. Pearson correlation coefficient was used to assess the correlation relationship between cardiovascular and metabolic disease risk markers with physical activity status and physical characteristics. A one-sample t-test was used to compare data from the present study to results reported in other comparable studies.

6.3 Results

6.3.1 Association between cardiovascular and metabolic disease risk markers and physical activity status in adolescents.

A total of 20 participants (12-17yr) (males n=12 and females n=8) gave consent to obtain blood samples and they also, completed their physical activity assessment. Based on the physical activity assessment using Puyau's cut-points, adolescents in the present study were grouped into normally active group (MVPA$\geq$300min.d^{-1}) (Male n=8, Female n=1) and low active group

(MVPA<150min.d^{-1}) (Male n=4, Female n=7). Physical characteristics of both groups are summarised in Table 6.2. The results showed that the normally active group was significantly older and taller than the low active group. Although there were significant differences between groups in body fat percentage and fat free mass (*P*=0.01, 0.01 respectively), there were no differences between groups in BMI or in percentile for age. As anticipated, the normally active group recorded a significantly higher predicted VO_{2max} than the low active group.

Table 6.2 Physical characteristics (means +SD) for 20 adolescents, (Active n= 9, Low active n= 11).

Physical characteristics	**Active**	**Low active**
Age (yrs)	15.7 ±1.3	14.0 ±1.6*
Mass (Kg)	60.2 ±6.6	53.6 ±10.2
Height (cm)	173.9 ±8.5	163.9 ±9.7*
BMI (Kg.m-2)	19.7 ±1.4	20.5 ±3.8
Percentile for age	49th ±23rd	64th ±32nd
Body Fat%	12.7 ±6.9	21.6 ±7.4**
Fat Free Mass (Kg)	52.5 ±6.9	41.8 ±7.8**
Mean arterial pressure (MAP) (mmHg)	83.1 ±6.3	81.6 ±8.7
Resting heart rate (bpm)	60.1 ±11.5	66.5 ±11.9
Predicted VO_{2max} (L.min^{-1})	3.4 ±0.9	2.6 ±0.8*

* Significantly different from the active group at *P* <0.05

* Significantly different from the active group at *P* ≤0.01

Physical activity data of the participants in each group is described in Table 6.3. The results showed that there were significant differences between normally

active versus low active adolescents in counts per minutes per day, moderate, MVPA and vigorous and very vigorous physical activity.

Table 6.3 Mean (±SD) of physical activity data for 20 adolescents (active n=9, low active n=11).

PA Parameters	All participants	Normal active	Low active
Number of Days	8.8 ±2.2	9.6 ±1.6	8.1 ±2.4
Registered Time (Min.day^{-1})	747.4 ±62.0	745.1 ±68.9	749.3 ±59.1
Counts.min^{-1}.day^{-1}	549.0 ±157.9	652.0 ±129.9	464.8 ±128.6**
Sedentary (Min.day^{-1})	609.9 ±63.0	597.7 ±65.4	619.9 ±62.3
Active (Min.day^{-1})	137.4 ±33.0	147.4 ±26.0	129.3 ±37.0
Light (Min.day^{-1})	106.6 ±30.4	102.0 ±30.2	110.3 ±31.5
Moderate (Min.day^{-1})	27.3 ±15.4	39.0 ±13.9	17.8 ±8.4**
Moderate to Vigorous (MVPA) (Min.day^{-1})	29.6 ±16.9	43.2 ±15.4	18.5 ±8.5**
Vig. & Very Vigorous (V&Vvig.) (Min.day^{-1})	3.6 ±5.4	6.4 ±6.7	1.3 ±2.4*

* Significant different from active at $P<0.05$

** Significant different from active at $P\leq0.005$

Blood samples of the 20 participants were analysed in the present study to investigate the association between cardiovascular and metabolic risk markers and physical activity status. Although the data appear to show that the normally active group have relatively higher values of total cholesterol and LDL and relatively lower value of HDL, there were no significant differences between groups in any of the traditional cardiovascular risk factors (cholesterol, triglycerides, HDL, VLDL and LDL) and all values fall within normal range expected (Figure 6.1).

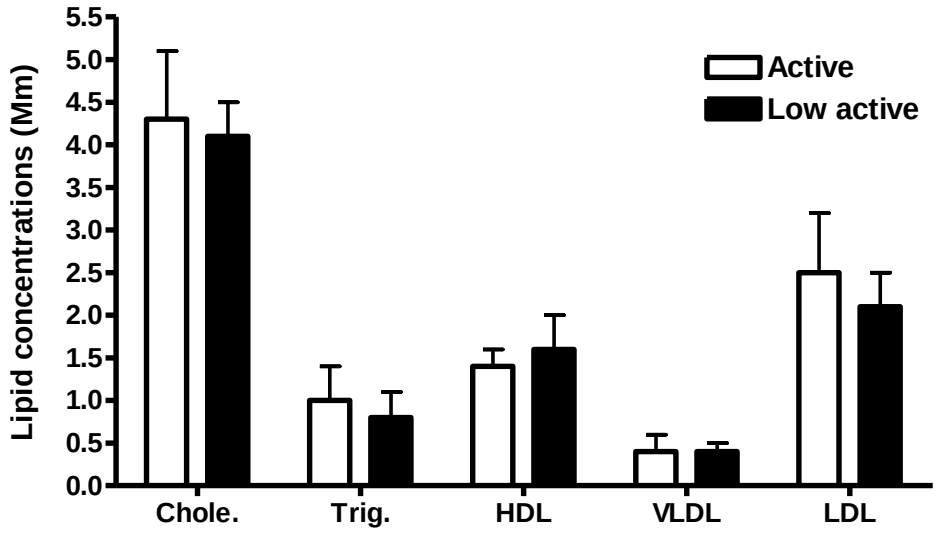
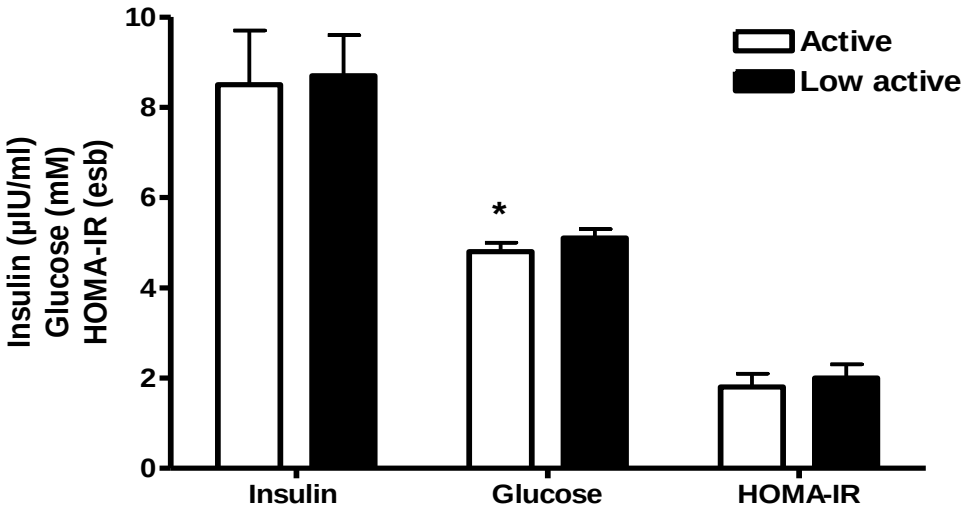


Figure 6.1 Mean ±SD of some of the lipid concentrations for adolescents (Active n=9, Low active n= 11).

Similar results were found between groups in some of the metabolic risk markers (insulin, glucose, HOMA-IR). Figure 2 illustrates that there were no significant differences between groups except in fasting glucose (*P*=0.03).



***** Significant differences from active group at *P*<0.05

Figure 6.2 Mean ±SD of some of the metabolic risk markers for 20 adolescents (Active n=9, Low active n= 11).

The results also revealed that there were no significant differences between groups in other cardiovascular and metabolic risk markers (adiponectin, IL-6, TNF-α, hsCRP, n-6:n-3 (ARA/EPA) and %n-3 HUFA/Total HUFA). Data in Table 6.4 appear to show that the normally active group have lower adiponectin and higher TNF-α and hsCRP but all were not significant. Leptin values were not obtained as all values were below the plate reader limit.

Table 6.4 Mean ±SD of some of the cardiovascular and metabolic risk markers for 20 adolescents (Active n=9, Low active n= 11).

Blood Parameters	Normal active	Low active
Adiponectin (µg/ml)	7.37 ±4.33	10.05 ±6.74
IL-6 (pg/ml)	6.18 ±1.77	7.87 ±7.80
TNF-α (pg/ml)	4.84 ±3.13	3.43 ±1.69
hsCRP (µg/ml)	1.16 ±1.42	0.34 ±0.17
n-6/n-3 (ARA/EPA)	9.29 ±4.85	11.15 ±4.21
% n-3 HUFA/Total HUFA	28.00 ±6.85	27.00 ±5.43

6.3.1.1 Correlations

The results of the correlation analyses between the cardiovascular and metabolic disease risk markers measured in the adolescents in the present study and physical activity status and physical characteristics are illustrated in Table 6.5. The results did not show a significant correlation between any of the blood markers and sedentary activity. However, there was a striking and strong significant negative correlation between active time and both fasting insulin and HOMA-IR. Time spent in MVPA was inversely correlated with both glucose and HOMA-IR. Similar to active time, a strong inverse significant correlation was found between vigorous and very vigorous activity time and both insulin and HOMA-IR. Furthermore, the predicted VO_{2max} was positively correlated with LDL and hsCRP.

Table 6.5 Correlation (r (CI), *P*) between the blood parameters versus the physical activity data ($min.d^{-1}$) for the Scottish adolescents in the present study (12-17yr, n=20).

Blood Parameters	**Sedentary**	**Active**	**MVPA**	**V&Vvgi**	**Predicted VO_{2max}** ($ml.kg^{-1}.min^{-1}$)
Cholesterol (mM)	(0.14) 0.56	(0.40) 0.08	(-0.03) 0.89	(-0.21) 0.37	(0.23) 0.32
Triglyceride (mM)	(0.10) 0.66	(-0.09) 0.72	(-0.10) 0.68	(-0.39) 0.09	(0.17) 0.47
HDL (mM)	(0.14) 0.56	(0.37) 0.11	(-0.08) 0.74	(0.11) 0.64	(-0.39) 0.09
VLDL (mM)	(0.10) 0.66	(-0.09) 0.72	(-0.10) 0.678	(-0.39) 0.09	(0.17) 0.47
LDL (mM)	(0.04) 0.88	(0.22) 0.36	(0.04) 0.86	(-0.19) 0.43	(0.44) 0.05*
MAP (mmHg)	(-0.05) 0.83	(0.06) 0.80	(0.06) 0.80	(0.14) 0.54	(0.21) 0.37
Insulin (µIU/ml)	(-0.13) 0.58	(-0.48) 0.03*	(-0.40) 0.11	(-0.47) 0.03*	(-0.27) 0.25
Glucose (mM)	(-0.10) 0.678	(-0.38) 0.10	(-0.45) 0.048*	(-0.23) 0.33	(0.28) 0.24
HOMA-IR	(-0.14) 0.57	(-0.52) 0.02*	(-0.45) 0.049*	(-0.46) 0.04*	(-0.31) 0.189
Adiponectin (µg/ml)	(-0.04) 0.87	(0.15) 0.53	(-0.04) 0.88	(0.07) 0.76	(-0.17) 0.48
IL-6 (pg/ml)	(0.12) 0.61	(0.10) 0.67	(-0.04) 0.87	(-0.03) 0.89	(-0.34) 0.15
TNF-α (pg/ml)	(-0.04) 0.86	(0.20) 0.39	(0.07) 0.78	(0.11) 0.64	(0.30) 0.20
hsCRP (µg/ml)	(-0.33) 0.58	(0.20) 0.41	(0.04) 0.86	(0.04) 0.88	(0.68) 0.00*
n-6/n-3 (ARA/EPA)	(0.55) 0.01*	(-0.14) 0.56	(-0.24) 0.33	(0.14) 0.56	(-0.38) 0.11
% n-3 HUFA/Total HUFA	(-0.54) 0.02*	(0.01) 0.97	(0.14) 0.57	(-0.10) 0.67	(0.20) 0.42

* Significant correlation at $P \leq 0.05$

** Significant correlation at $P \leq 0.001$

On the other hand, the correlation results between blood markers and body characteristics showed that there was a significant negative correlation between HDL and age, weight, height and fat free mass (r(CI) *P* value) ((-0.48) 0.03, (-

0.57) 0.01, (-0.55) 0.01 and (-0.60) 0.01 respectively). A significant positive correlation was found between adiponectin and body fat percentage ((0.45) 0.05) and a negative correlation was observed between IL-6 and BMI percentile for age ((-0.46) 0.04).

6.3.2 Comparison between the results of the present study to the data of other studies in children and adolescents.

The blood parameters data of all adolescents in the present study (20 adolescents) were compared to the mean (±SD), mean (±SE) or mean (95% CI) of the blood parameters in other studies. The comparison is presented in Table 6.6. Some of these studies assessed physical activity status using subjective measures such as questionnaire (Medina-Urrutia *et al.*, 2008; Platat *et al.*, 2006; Rubin *et al.*, 2008) whereas others used objective devices such as the Actigraph to assess the habitual physical activity (Nguyen *et al.*, 2010; Brage *et al.*, 2004). The results showed that there were significant differences in some of the cardiovascular and metabolic disease risk markers between the data of the adolescents in the present study versus the data found in other studies. Particularly, there were significant differences between the present study and the studies that used subjective measures to assess physical activity (Table 6.6 a,b,c) in cholesterol, HDL, insulin, HOMA-IR, adiponectin and TNF-α with all but adiponectin being higher in the present sample compared with the data from those studies. On the other hand, the results of the comparison between the data of the present study and the data in the studies which also used objective

measures (Table 6.6 d,f) showed significant differences between the compared data with lower plasma triglyceride and higher fasting insulin and fating glucose (*P*=0.00, 0.02 and 0.00 respectively) in the present study.

Table 6.6 Comparison between the mean (±SD) of the cardiovascular and metabolic disease risk markers of the participants in the present study versus similar data in other studies.

Blood Parameters	**The present study** **n=20**	**Other studies** [a b c] **Use questionnaire**	**Other studies** [d f] **Use Actigraph**
Cholesterol (mM)	4.15 ±0.59	3.67 ±0.48 [a *]	NA
Triglyceride (mM)	0.87 ±0.33	0.90 ±0.28 [a]	1.12 (1.1, 1.2) [d **]
HDL (mM)	1.50 ±0.34	1.21 ±0.19 [a §]	1.46 (1.4, 1.51) [d]
LDL (mM)	2.25 ±0.55	2.13 ±0.42 [a]	2.39 (2.3, 2.4) [d]
Insulin (µIU/ml)	8.60 ±1.00	5.30 ±4.10 [a §]	8.02 ±4.70 [f *]
Glucose (mM)	4.96 ±0.22	5.03 ±0.29 [a]	4.72 (4.7, 4.8) [d §]
HOMA-IR	1.90 ±0.27	1.20 ±1.00 [a §]	NA
Adiponectin (µg/ml)	8.85 ±5.80	14. 01 ±4.73 [b §]	NA
IL-6 (pg/ml)	7.11 ±5.84	6.60 ±0.60 [b]	NA
hsCRP (µg/ml)	0.71 ±1.02	0.63 ±0.09 [b]	NA
TNF-α (pg/ml)	4.10 ±2.51	1.34 ±0.75 [c §]	NA

* Significant different from the present study's value at *P*<0.05

** Significant different from the present study's value at *P*<0.005

§ Significant different from the present study's value at *P*≤0.001

a Medina-Urrutia *et al.* (2008), n=39, lean male and female age=13.3 ±0.9

b Platat *et al.* (2006), (all values in mean ±SE): n=210, lean male and female, age= 11.5 ±0.02

c Rubin *et al.* (2008), n=60, lean male and female, age=(10-14yr)

d Nguyen *et al.* (2010), all values (95% CI) n=617, lean male and female, age=13.9 yr (13.7-14.2)

f Brage *et al.* (2004), n=589, lean male and female, age=(9.6±0.4)

6.4 Discussion

6.4.1 Association between cardiovascular and metabolic disease risk markers and physical activity status in adolescents.

The results of the present study have not surprisingly indicated that there are significant differences between normally active versus low active adolescents in count per minute per day, but there are also differences in MVPA and in vigorous and very vigorous activity as well as in predicted VO_{2max}. Cardiovascular and metabolic disease risk markers were not significantly different between groups except for fasting glucose but there was a strong association between active time, MVPA, and vigorous and very vigorous time with glucose, insulin and HOMA-IR which indicates the potential for signs of progression to metabolic disease already being present in this sample.

Based on the published evidence of the normal range of the traditional cardiovascular and metabolic disease risk markers in adolescents (cholesterol, HDL, VLDL, LDL, triglyceride, insulin, glucose and HOMA-IR), all the traditional blood markers in the present study were within the normal range (Medina-Urrutia *et al.*, 2008; Huang *et al.*, 2007; Andersen *et al.*, 2003; Steinberger *et al.*, 2009; Andersen *et al.*, 2006). Although some studies have found a positive effect of physical activity on some of the cardiovascular and metabolic disease risk markers (Brage *et al.*, 2004), Hallal *et al.* (2006) in a systematic review concluded that physical activity level in adolescents had no consistent effect on lipid concentrations, glucose and blood pressure. The balance of omega 3 and omega 6 in high unsaturated fatty acids (HUFA) has been investigated as an

inflammation marker and good predictive index for cardiovascular diseases (Thorseng *et al.*, 2009; Lands, 2003).

In the present study there were no significant differences between normally active versus low active groups in the n-6:n-3 (ARA:EPA) ratio and the percentage of n-3/ total HUFA. Furthermore, both groups had normal level of n-6/n-3 (ARA/EPA) comparing with the published values in healthy children (ARA/EPA=16.96) (Bell *et al.*, 2009). In terms of %n-3 HUFA/total HUFA, adolescents in the present study had similar values to those observed in adults (Chapter 4) and not far from a previous finding in adults (Marangoni *et al.*, 2007). However, all values of %n-3 HUFA/total HUFA reported in the current study were lower than the recommended levels by Ideal Omega Test provided on online (Ideal Omega test (no publication date). Available: http://www.idealomegatest.com).

On the other hand, there was a fairly strong association between sedentary behaviour and the examined fatty acid indexes. The association was positive with n-6/n-3 ratio and reversed with the %n-3 HUFA/total HUFA. Such results may alert that sedentary and low active adolescents may be at risk of not having the minimum level of the good fatty acid (omega 3) and/or high level of undesirable fatty acid (omega 6) but this is most probably through differences in dietary intake.

The present study is the first study when examined the association between some of the important fatty acids indexes and physical activity levels using objective measures. Thus, more studies investigating the indirect effect of

physical activity intensity on the fatty acids components in adolescents may be beneficial.

Likewise, other cardiovascular and metabolic risk markers (adiponectin, IL-6, TNF-α and hsCRP) were within the range of other apparently healthy adolescents in different studies (Rizzo *et al.*, 2008; Huerta, 2006; Eleftheriou *et al.*, 2008). There are few studies that have examined the effect of physical activity status on these cytokines. Rubin *et al.* (2008) investigated the influence of vigorous physical activity on key cytokines in adolescents using a questionnaire. They found that there were no significant differences between boys with high vigorous physical activity versus boys with low vigorous physical activity in all cytokines including adiponectin, IL-6 and TNF-α whereas girls with high vigorous physical activity had higher adiponectin than girls with low vigorous physical activity (P=0.01) but there were no significant differences between girls (high versus low vigorous physical activity) in other cytokines including IL-6 and TNF-α. Although the physical activity was objectively measured in the present study, the strength of the results may be affected by the small sample size.Thus, in order to investigate the influence of physical activity status on these blood markers precisely, the sample size may need to be increased and/or a wider range of physical activity status studied such as very active versus completely sedentary adolescent groups.

The second explanation for the absence of the effect of physical activity status on the blood risk markers in the present study is the body composition. All adolescents in the current study were lean and quite active despite not meeting the physical activity guidelines. In chapter 4, the influence of body adiposity on

the cardiovascular and metabolic risk markers in adults were greater than physical activity but higher intensity activity also had important associations with lower body fat. More investigation may be needed in adolescents with a larger sample size and a wide range of physical activity to investigate this relationship further in a younger age group.

6.4.1.1 Correlation.

The main aim of the present study was to investigate the association between cardiovascular and metabolic disease risk markers and physical activity status. Therefore, correlations were calculated to determine the association between these blood markers and physical activity levels as well as with the body composition of all adolescents (total n=20, male=12, female=8). Table 6.3 indicated that the mean of MVPA of all adolescents was about 210 $min.w^{-1}$ which is less than the guidelines average ($\geq$300$min.w^{-1}$).

The results of the correlations indicated that the association between physical activity and the blood markers of the cardiovascular and metabolic diseases were absent except for some of the key metabolic risk markers (insulin, glucose, fatty acids indexes, HOMA-IR, LDL and hsCRP). In a previous study, Brage and colleagues (2004) investigated the association between the features of the metabolic syndrome and an objectively measured physical activity and fitness in 589 Danish children (9.6yr ±0.4). The average physical activity intensity was assessed as total counts per registered time. They found that physical activity of the children (mean ±SD, 660±233 cpm) was inversely

associated with insulin (*P*=0.02) and also inversely associated at borderline significantly with triglyceride (*P*=0.05), but not with glucose (*P*=0.12) or HDL (*P*=0.17). In adolescents, a review of the literature by Twisk (2001) concluded that short-term physical activity in adolescents had no effect on lipid concentrations, blood pressure or glucose concentrations but there was a positive association with HDL (Twisk, 2001). Similar findings in adolescents were found in other cross-sectional studies as physical activity was found to be associated with insulin sensitivity (Schmitz *et al.*, 2002), and improved HDL (DeFronzo *et al.*, 1975). On the other hand, the present study is the first study to investigate the association between some of the fatty acids indexes and physical activity levels in apparently healthy lean adolescents using objective measures. Although all participants in the current study were not highly active, there were a fairly strong association found between fatty acids indexes and sedentary lifestyle.

These results raise a question whether the sedentary adolescents do not eat enough omega 3 and too much omega 6? In contrast with the finding in adults (Chapter 4), the association between the examined fatty acids indexes and vigorous physical activity were absent in adolescents in the present study. These results are probably because the range of the physical activity levels of the adolescents in the current study were not wide enough. The range was between low active and normal active but not between sedentary and highly active. Actually, there are no similar studies to compare these finding with.

Findings of the current study agree with most of the results of some of the previous studies (Brage *et al.*, 2004; Schmitz *et al.*, 2002). However, there were

some unanticipated findings which were the positive association between the estimated VO_{2max} versus each of LDL and hsCRP. These findings may be as a result of a recent infection which may affect some of the blood test results (hsCRP) and diet habits that may contain over limit of fatty food (LDL). Age differences between groups may affect the results as the active group were 2 years older than low active group. In addition, the small sample size of the present study may lead to an unexpected result. Furthermore, the differences between physical activity statuses of participants were not large enough such as high active, active, low active to sedentary participants. Moreover, diet may play a role especially in the absence of high levels of physical activity (e.g. MVPA $\geq$1h.d^{-1} or an accumulation of $\geq$420min.w^{-1}).

All adolescents were lean and had the normal range of these blood markers; therefore, small differences between them in the estimated VO_{2max} may not reflect the real association between the estimated VO_{2max} and LDL and hsCRP. Thus, the unanticipated positive correlation between VO_{2max} and LDL and hsCRP may come by chance and more consideration should be taken for further studies such as sample size and wider range of physical activity.

On the other hand, the association between physical activity status and adipokines and inflammatory markers (adiponectin, IL-6, TNF-α and hsCPR) were absent except the previously mentioned positive association between the estimated VO_{2max} and hsCRP. Some of the recent studies could not agree on the association between physical activity and some of the key adipokines (adiponectin, IL-6, TNF-α and hsCPR) and others also did not find these associations (Ischlander *et al.*, 2007; Isasi *et al.*, 2003; Platat *et al.*, 2006;

Moran *et al.*, 2005). For example, Isasi *et al.* (2003) investigated the association of physical fitness with CRP in children and young adults (6-24yr, >85% of them <15yr). Physical work capacity at a heart rate of 170bpm (PWC_{170}) protocol was used to assess the physical fitness. They found that physical fitness was higher in boys than girls but hsCRP level were not significantly different. Even though, physical fitness was inversely associated with CRP in all participants as a group (both gender) (r=–0.22, P<0.01) and in boys (r=–0.32, P<0.01) but not in girls (r=–0.15, P>0.05). In another study by Moran *et al.* (2005), they found no significant association between physical activity assessed by a questionnaire and CRP in 342 healthy youth 10-16yr.

Similarly, the association between physical activity and plasma adiponectin and IL-6 concentration was not clear. Data from 640 children (mean ±SE, 11.53 ±0.02) showed that physical activity was inversely associated with IL-6 but not with adiponectin (Platat *et al.*, 2006). Conversely, Ischander *et al.* (2007) concluded that after controlling for percent body fat, TNF-α was significantly associated with physical activity whereas IL-6 was no longer significant related to physical activity in lean female adolescents. In fact, there are different factors that may affect the cardiovascular and metabolic disease risk markers including physical activity status. Most of the previous studies used subjective physical activity measures such as questionnaire. In a systemic review, Hallal *et al.* (2006) concluded that although there are some studies that have investigated the influence of physical activity in adolescents on these blood risk factors, physical activity in adolescents may reduce adult morbidity even though it does not appear to affect risk markers measurable during adolescence itself. They

also added that the definition of physical activity recommendations for adolescents based on the short-term benefits on health is not possible at this stage, and the health benefit seems to vary according to the diseases (Hallal *et al.*, 2006). Thus, the association between cardiovascular and metabolic risk markers and physical activity status may need to be investigated precisely with considering sample size, the most appropriate definition of the physical activity levels, adequate objective instruments with the appropriate methods and all other related factors that may play role in influencing these markers including body composition, diet, and socioeconomic factors. Nonetheless, there was evidence of an association between activity intensity and metabolic disease that suggests that early signs of disease can be detected in this age group.

6.4.2 Comparison between the results of the present study to the data of other studies in children and adolescents.

The second aim of the current study was to compare the results of the present study with the results in similar studies. Data of the total adolescents (n=20) were compared to other similar studies (Medina-Urrutia *et al.*, 2008; Platat *et al.*, 2006; Rubin *et al.*, 2008; Nguyen *et al.*, 2010; Brage *et al.*, 2004). Based on the data shown in Table 6.1, all adolescents in the present study (n=20, males=12, females=8) appear to be healthy lean and fairly active (resting HR, predicted VO_{2max}), although they do not meet the recommended guidelines for MVPA as assessed using Puyau' cut-points. The first comparison was with a study by Medina-Urrutia *et al.* (2008). In this study participants were grouped

into four groups. The group of which they had normolipidemics (n=39) was selected as a sample with normal range. There were significantly lower values in some of the compared blood markers between Medina-Urrutia's study and the present study. This is most likely due to the sample selection method as from 315 participants, only 106 adolescents were selected based on their lipid-lipoprotein concentrations and grouped into 4 groups. Group 1 was the participants with low HDL and high triglyceride (n=16). Group 2 was the participants with only low HDL (n=31). Group 3 was the participants with only high triglyceride (n=20). Only 39 participants were selected for group 4 as normolipidemics from the remaining 248 participants whom selected for this study without giving any justification about wither the 39 the only participants with the normal criteria or they were selected because they have the low average range of the blood lipids. Thus, one explanation for the significant differences between this study and the present study is probably they (the 39 participants with normolipidemics) had low concentration of blood lipid markers. In addition, Medina-Urrtia did not mentione full data about their participants' physical activity rather than a pilot programme to promote a healthy diet and regular practice of physical exercise in the public sample (junior high school).

The second study that was selected to be compared to the present study is a study investigated by Platat *et al.* (2006). They examined the relationship of physical activity with metabolic syndrome features and low-grade inflammation in adolescents (n=210) including boys and girls aged (mean) 11.5yr using a questionnaire for assessing physical activity status. There were no significant differences between the Platat's study and the present study in IL-6 or CRP.

However, there was a strong significant difference between studies in adiponectin (*P*=0.001). Regardless of the significant differences between Platat's study and the current study in adiponectin, both studies agree that the association between physical activity and plasma adiponectin were absent and these results consistent with other studies examined the effect of exercise training programme on adiponectin concentration (Hulver *et al.*, 2002; Houmard *et al.*, 2000). It appears that adiponectin was affected by body composition (adiposity) in adults (chapter 4) and in adolescents (Weiss *et al.*, 2004), but the effect of physical activity status may need to be examined with considering all other factors such as diet, socioeconomic and body adiposity that may play a role in preventing the accurate and actual results (Platat *et al.*, 2006; Nguyen *et al.*, 2010; Volek *et al.*, 2005).

The result of the TNF-α concentration in the current study was compared with the similar data from a study by Rubin *et al.* (2008). They examined the association between vigorous physical activity and cytokines in 60 adolescents (age=10-14yr). Although there was a significant difference between the present study results in TNF-α and Rubin's study, both studies failed to find significant association between physical activity and the TNF-α concentrations even in vigorous physical activity. Moreover, both studies had large variability in TNF-α value. Similar to the other pro-inflammatory cytokines (adiponectin), Rubin and colleagues (2008) found that overweight adolescents had higher TNF-α concentration. The only study –to date – that found association between physical activity and TNF-α concentration was in lean sedentary versus lean active female (Ischlander *et al.*, 2007). This result was probably due to the size

of the sample as they had 37 participants in each group. All the three studies (Table 6.6 a,b,c) that compared to the current study used subjective measures to assess physical activity status. Therefore, more similar studies that assessed the habitual physical activity using objective measures were searched and compared to the results of the present study.

Apparently no studies have investigated the association between the habitual physical activity and the key adipokines markers as well as the traditional cardiovascular risk markers in apparently healthy lean adolescents. However, there is a study that examined the association between physical activity using objective (Actigraph GT1M) and metabolic syndrome including triglyceride, HDL, LDL, insulin and glucose in lean Vietnamese adolescents (mean, 13.9yr) (Nguyen *et al.*, 2010). In this study, Nguyen and colleagues set the Actigraph in 1min epoch and they calculated the average time spent per day in MVPA ($\geq$3.0MET) using Trost's cut-points (Trost *et al.*, 2002). The results showed that the participants were active (60.3 $min.d^{-1}$), though, they had significantly higher value in triglyceride than the adolescents in the present study. On the contrary, the Vietnamese adolescents in Nguyen's study had significantly lower concentration of fasting glucose. There were no significantly differences between the studies in the other blood lipid markers, HDL and LDL concentrations. However, these blood lipids markers concentrations were relatively more favourable in the present study than Nguyen's study. Although, there were some differences between some of the metabolic syndromes between the compared studies, Nguyen's study assessed the habitual physical activity using inappropriate cut-points and period epoch. For more details about

some evidence of the most appropriate use of Actigraph's cut-points and period epoch for children and adolescents is available in chapter 5.

The second study, by Brage *et al.* (2004) investigated the association of the metabolic syndromes features with objectively measured physical activity using Actigraph device in 589 Danish boys and girls (9-10yrs). From this study, only insulin data was selected to be compared to the insulin data in the present study. Participants in both studies were quite active and the average of the counts per minutes were not significantly different between the present stud versus Brage's study (549 ±158 vs. 600 ±211, *P*=0.165 respectively). Despite the significant differences shown in Table 6.6 between the insulin concentrations in both studies, both insulin concentrations were within normal average range.

Given all these outcomes from the different studies that investigated the relationship between physical activity status and cardiovascular and metabolic disease risk markers in apparently healthy lean adolescents, the association between physical activity and these key blood markers were still unclear. One of the reasons probably because these blood markers are more likely to be affected by a contribution of different factors such as socioeconomic status (Platat *et al.*, 2006; Nguyen *et al.*, 2010), diet (Volek *et al.*, 2005), body adiposity (Steele *et al.*, 2008) as well as physical activity status. Therefore, the influence of physical activity status on such key blood markers maybe become more clear in the case of sedentary versus highly active adolescents (e.g. MVPA $\geq$1hr.d^{-1}) because there are many studies including the present study demonstrated that even in quite active but apparently healthy lean adolescents,

the cardiovascular and metabolic risk markers were within normal average range (Isasi *et al.*, 2003; Platat *et al.*, 2006; Nguyen *et al.*, 2010; Brage *et al.*, 2004; Ekelund *et al.*, 2009). Moreover, Ekelund and colleagues (2009) concluded that even small increases in physical activity may significantly reduce the risk of metabolic syndromes in healthy children. Thus, more investigation may be needed with considering the limitations of the previous studies including the control of all factors that may play roles in affecting these key blood markers in order to clarify the actual association between physical activity status and the cardiovascular and metabolic disease risk markers. In turn, consider the results in the physical activity recommendations for adolescents to improve the public health promotion (Hallal *et al.*, 2006).

The present study was well controlled in terms of procedures and design. Due to the time limit given to complete the current study, the sample size was below the required number. In turn, the results of the present study would be more conclusive if the sample size was equal to or more than the required number for each group (>20 participants in each group). Although the current study can be considered as a pilot study, the results have provided some valuable information about the association between physical activity and cardio-metabolic disease risk markers.

6.5 Conclusion

In conclusion, some of the cardiovascular and metabolic risk markers found in Scottish adolescents in the present study (insulin, HOMA-IR and TNF-α) were

significantly higher than the matched blood risk markers from adolescents in other studies (Medina-Urrutia *et al.*, 2008; Platat *et al.*, 2006; Rubin *et al.*, 2008; Nguyen *et al.*, 2010; Brage *et al.*, 2004). These were also associated with physical activity status suggesting that in this sample of Scottish adolescents there appears to be signs of movement towards higher fasting insulin and HOMA-IR that could progress if physical activity is not increased to meet the recommended physical activity guidelines for adolescents. Thus, policy makers should give a priority to enhance Scottish adolescents in high schools to perform physical activity and meet the recommended guidelines for adolescents. Further studies are needed to investigate the relationship between physical activity status and cardiovascular and metabolic disease risk markers with considering the limitations of the previous studies.

Chapter 7. General discussion

The present studies (chapter 2 – chapter 6) have produced a body of descriptive data that along with previous work provides some further knowledge and understanding of the relationships between physical activity and health. The contribution of the present studies, includes assessment of the reliability and validity of the new generations of some of the popular instruments, use of objective measurements to track habitual free-living physical activity in Scottish adults and adolescents (12-55yr), and investigates the associations between habitual physical activity status, adiposity, and cardiovascular and metabolic disease risk markers.

7.1 Reliability and validity of Actigraph, ActivPAL and SenseWear PRO$_2$

One of the first aims of the present thesis was to assess the reliability and validity of the new generations of Actigraph (AG), ActivPAL (AP) and SenseWear PRO$_2$ (SW) measurement devices in adults (18-55yr), and in adolescents (12-17yr), using experimental laboratory work.

Reliability

In the first study (chapter 2), reliability was evaluated by comparing the output of the accelerometry devices (as estimated energy expenditure (EE)) between the first and second tests. All accelerometers (AG, AP and SW) showed strong

intra-instrument reliability for assessing EE on the flat and on a 5% gradient in both lean and overweight/obese participants, across all of the walking speeds tested. These findings are consistent with other findings in the literature which have evaluated the reliability of the same model such as in AP (Ryan *et al.*, 2006), previous models of AG (model MTI 7164) (Welk *et al.*, 2007) or previous software versions for the SW device (software version 1.0) (Fruin and Rankin, 2004). Thus, ActiGraph, ActivPAL and SenseWear can be deemed to be instruments that are reliable in their assessment of activity energy expenditure during different walking speeds on flat surfaces and on a 5% gradient in both lean and overweight/obese adults.

The adolescents group, similar to adult groups, all accelerometer devices (AP, SW and AG) were reliable with strong intra-instrument reliability across all speeds in both gradients. The first study was the first available study that examined the reliability of AP in assessing EE during walking in adolescents (12-17yr). There are very few studies that have examined the reliability of AP in assessing EE in children or adolescents (Ryan *et al.*, 2006). Even though, the previous studies examined the reliability of AP in steps counts and/or physical activity in relation to posture. The reliability of the other devices (SW and AG) has been examined previously in children and adolescents (Freedson *et al.*, 2005; Liden *et al.*, 2001). All the previous studies including the first study agreed that all these movement sensing devices (PA, SW and AG) were reliable in assessing light and moderate locomotors physical activity such as walking in different pace. The contribution of the first study, in this regard, can be found in two aspects. The first was that it is the first study that examined the

reliability of AP in assessing EE during walking on the flat and at 5% gradient in adolescents (12-17yr). The second addition was that examining the reliability of the new generations of these devices including the software version used with these devices in adolescents. Therefore, all the examined accelerometer devices (AP, SW and AG) were reliable with strong intra-instrument reliability across all speeds in both gradients in adolescents (12-17yr) but reliability is only part of the requirement of a valuable instrument for use with the general population.

Validity

The validity of the three accelerometry devices was assessed by comparing the estimated activity EE obtained from the accelerometer prediction equations with indirect calorimetry. Although the AP has been shown to be valid in adults when measuring the time spent in static activities such as sitting/lying and standing, and during dynamic activity such as stepping (steps.min^{-1})) (Dahele *et al.*, 2007; Godfrey *et al.*, 2006; Koulouri *et al.*, 2006; Godfrey *et al.*, 2008), there is no previous study that has assessed the validity of AP in estimating habitual physical activity EE in apparently healthy lean and overweight/obese individuals. The observations made in the first study of the present thesis suggest that in apparently healthy adults, AP does not provide a valid assessment of activity EE during physical activity due to its low sensitivity to detect changes in activity intensity.

The SenseWear PRO$_2$ (SW) device showed some valid EE estimations and more than AP when assessing EE during walking. The SW was valid in

assessing EE during brisk walking (6Km.h^{-1}) in lean adults on the flat, as well as during slow walking (3Km.h^{-1}) on a 5% gradient in lean and overweight/obese participants. Moreover, the SW was more sensitive than the AP at detecting EE differences between all speeds for both groups on both gradients. These results are probably due to the fact that the SW device estimates activity EE by integrating mechanical and physiological components including a 2-axis accelerometer, a heat flux sensor, a galvanic skin response sensor, a skin temperature sensor, and a near-body ambient temperature sensor (Liden *et al.*, 2001). Furthermore, the SW device assesses activity EE based on information provided about the user such as age, gender, height and weight. However, a key limitation in SW appears to come from the logarithm that is used in the software. Version 6.1 used in the first study showed more accuracy than the old version such as 1.0 (Fruin and Rankin, 2004). However, the SW device still had some problems in assessing activity EE in adults during walking especially on uphill surfaces. This is somewhat surprising given the multiple physiological inputs used by the device to estimate activity EE. Thus, more studies may be needed in order to improve the algorithms for the software in order for it to assess activity EE more accurately.

The third accelerometer used in the first study was the new generation of ActiGraph GT1M (AG). The AG is a locomotor-based uniaxial accelerometer, smaller and cheaper than both SW and AP. Nevertheless, AG showed more accurate activity EE assessment during walking on the flat than SW, especially in lean adults. The validity of the previous model of AG (MTI 7164) has been evaluated in terms of EE assessment during walking at different speeds

(Freedson *et al.*, 1998; Hendelman *et al.*, 2000; Crouter *et al.*, 2006a). Part of the contribution of the first study was also to examine how accurate Freedson's equation (Freedson *et al.*, 1998) is for prediction of EE in lean and overweight/obese adults under different walking conditions using the new generation of AG (GT1M), and it seems that Freedson's equation is more applicable to lean adults based on the observations reported. One of the advantages of the new generation of AG (GT1M) over SW (with using software version 6.1) is that AG showed a significantly higher correlation with indirect calorimetry at each speed separately in both groups (lean and overweight/obese adults) on both gradients. Although some activity EE as a result of physical activities are unlikely to be accurately measured by AG (such as highly static exercise, carrying a load or walking uphill), AG can still be viewed as the most sensitive instrument to assess physical activity intensity levels during habitual physical activity (Matthew, 2005).Therefore, the equation used for AG to predict activity EE requires development so that it can provide more accurate estimation of activity EE during different types of physical activity and in overweight/obese adults.

Overall, AG (GT1M) provided more accurate EE estimates compared to the other devices when assessing EE during walking in lean adults at normal and brisk walking pace. Moreover, AG and SW were able to detect EE differences between walking speeds for both groups on both gradients whereas AP was only able to detect EE between the slow and fast speeds for lean participants. Thus, all these devices (AP, SW and AG) and the software or logarithms provided with them needs to be developed through further study so that they

can more accurately assess physical activity EE. AG is the most commonly used objective measure for physical activity assessment and is very easy to use in research. Therefore, a strong suggestion is made to improve the new generations of the AG device as well as to develop the equations used on AG data in order to better estimate physical activity EE generated by people including lean and overweight/obese adults. These developments are required if any useful energy intake versus energy expenditure comparisons are to be made that would add real value to this cheap objective measure of physical activity. Better estimates of free-living activity EE from simple devices would be a big step forward in tracking energy balance more precisely without the need to adopt far more costly alternatives such as doubly labelled water.

In healthy adolescents, validity of the three movement sensing devices (AP, SW and AG) in assessing EE during walking at different speeds on the flat and on an incline (5% uphill) were examined. Similar to adults, all accelerometers had problems with validity in adolescents. On the flat, none of the accelerometers were valid to assess EE during walking speeds in adolescents except AP during walking at 3Km.h^{-1}. AG and AP both underestimated activity EE on the flat across all speeds whereas SW overestimated activity EE. On an incline, SW was the only device that can predict EE during normal walking 4.5Km.h^{-1} as well as at overall speeds for each gender groups.

Data in the first study (chapter 2) was the first study that investigated the validity of AP in estimating EE during walking in different speeds on flat and at 5% gradient. The results from AP are probably due to the equation that is built into the AP's software programme which is for adults and cannot be modified for

children or adolescents. It is well known that energy cost in adolescents is changeable as body growth / maturation occurs and also children have higher energy cost of activity compared to adults (Freedson *et al.*, 2005). AP has been found to be reliable and valid in terms of assessing step count and cadence during walking (Ryan *et al.*, 2006). Most of the studies that use AP have been on adults and were measuring steps and cadence (steps.min^{-1}) as well as the time spent in static posture activities (sitting/lying and standing) and dynamic activity (stepping and cadence) (Dahele *et al.*, 2007; Godfrey *et al.*, 2006; Koulouri *et al.*, 2006; Godfrey *et al.*, 2008). However, the data obtained in this thesis indicates that AP is unable to detect the EE differences between walking speeds in adolescents as a whole group or in each gender on both gradients studied except between the slow and brisk walking speeds. To date, there is a lack of studies that have investigated the validity of AP in assessing EE in children and adolescents. Therefore, AP as a small and relatively cheap device needs to be developed for the purpose of assessing physical activity EE in adolescents.

Although SW uses integration of a 2-axis accelerometer with other sensors such as a heat flux sensor, galvanic skin response sensor, and skin temperature sensor, as well as users data such as age, gender, height and weight, SW was not able to accurately assess EE during walking on the flat or at 5% incline in adolescents. Previous studies including a study using the same software version 6.1 in children (10.6 ±1.2yr) demonstrated that there was an overestimation or underestimation of the activity EE measured during physical activity that included walking at different speeds (Mealey, 2008; Arvidsson *et*

al., 2007; Dorminy *et al.*, 2008). Thus, more studies may be required in order to improve the logarithm used in the SW software for assessing physical activity EE more accurately in children and adolescents.

In adolescents, AG, with Freedson's equation for young adults, was not valid in assessing EE during walking at different speeds. Freedson's equation for adults (Freedson *et al.*, 1998) was selected because the first study used the same walking speeds and gradient for both adults and children. The second reason was that the mean predicted VO_{2max} ($ml.kg^{-1}.min^{-1}$) of the adolescents in the first study was not significantly different from lean adults (P= 0.17). The data indicated that AG and use of the Freedson's equation was not accurate in assessing EE during walking even on the flat in adolescents. Although AG was sensitive enough to detect the differences between speeds on both gradients in adolescents, Freedson's equation that was developed based on lab testing procedures was not applicable on adolescents (~15yr) even if they had VO_{2max} close or similar to the adult's values. This would suggest that body mass is likely to be the key factor influencing the validity of this equation for use with adolescents. Bassett *et al.* (2000) conclude that "no single regression equation appears to accurately predict energy expenditure based on acceleration score for all activities". Until appropriate methods of data collection, processing and interpretation of AG outcomes are established to standardise use of AG, caution should be taken when using published equations for AG counts in an attempt to assess PAEE (Freedson *et al.*, 2005). The contribution of the first study was therefore to demonstrate that Freedson's equation that developed for adults was not applicable to adolescents for estimating physical activity EE.

Furthermore, this data suggests that the use of counts per minute outputs from AG to identify activity intensities is the most appropriate use of this device in adolescents.

In general, these accelerometers were deemed reliable for both adults and adolescents during walking speeds on both gradients. However, more research is required in order to improve the ability of these devices to accurately estimate physical activity EE.

7.2 Health benefits of physical activity status in adults (lean and overweight/obese)

There are wide ranging benefits to be gained from engaging in sufficient physical activity (Lee *et al.*, 1999; Blair and Brodney, 1999; Ekelund *et al.*, 2009). However, the health benefits of physical activity assessed using objective physical activity measurements such as accelerometry combined with investigation of some of the key cardiovascular and metabolic disease risk markers may aid our understanding of the health benefits related to habitual physical activity intensity. Adding to previous studies, the second study of this thesis (Chapter 3) assessed the habitual physical activity of adults using objective measurements, and the third study (Chapter 4) went on to investigate the associations between physical activity status and some of the key cardiovascular and metabolic disease risk markers. The outcomes of these studies are discussed in the next section.

7.2.1 Physical activity assessment in adults (lean and overweight/obese) using objective measures

In the second study (Chapter 3), the habitual physical activity of 55 adults including lean (n=33) and overweight/obese (n=22) participants was assessed objectively using 1 min and 10 min bout activity criteria. There were two main aims of the study. The first was to assess the habitual physical activity of healthy lean and overweight/obese adults using objective measurement (and the new generation of AG (GT1M)). The second aim was to assess the agreement between the habitual physical activity of the research groups (lean & overweight/obese) with the recommended guidelines for physical activity in adults published by ACSM (2007), SHeS & HEPS (2008) and DHHS (2005).

Although the 1 min bout method may be beneficial for studying adult activity habits, performing a recommended minimum of 10mins per bout has been reported to have a clearer association with waist circumference and BMI changes over a period of time (Strath *et al.*, 2008). The results from the study reported in Chapter 3 of this thesis indicated that there were no significant differences in habitual physical activity between lean and overweight/obese groups except in the percentage of time spent in vigorous and very vigorous physical activity (for both 1min bout and 10min bout methods). The results agree with some previous studies. For example, Cooper and colleagues (2000) concluded that non-obese adults spent significantly more time in MVPA intensity than obese, but only on weekends and there were no significant differences between groups on weekdays. Other studies using accelerometry found that there were no significant differences in activity counts between lean

and obese groups (Meijer *et al.*, 1992). The absence of differences between groups in MVPA in the second study (Chapter 3) could be because some of the overweight/obese participants were active (>73% of overweight/obese participants spent MVPA $\geq$30min.wk^{-1}) as most of them engaged in an exercise programme for health and/or weight loss. On the other hand, some of the lean participants were not physically active enough (>20% of lean participants engaged in MVPA <30min.wk^{-1}). Another possible reason could be that some of overweight/obese participants maybe tried to show that they were physically active when their activities were being monitored (Lightman *et al.*, 1992; Jakicic *et al.*, 1998). However, a key factor could be that more vigorous activity may help to regulate body fat than lower intensity activity given that this was the only discernable difference between lean and obese groups in Chapter 3. Interestingly, Tremblay and colleagues (1994) concluded that vigorous intensity exercise was found to be associated with greater fat loss assessing using change in sum of skin-folds, than exercise of low to moderate exercise intensity. Although there were no significant differences between groups in the time spent in MVPA, lean participants spent significantly more time in vigorous and very vigorous activity than overweight/obese participants in Chapter 3. In turn, vigorous activity could be considered beneficial for body fat regulation in the population and requires further study.

Regardless of the comparison between lean versus overweight/obese adults, both groups were doing enough physical activity (even when 10min bouts method was used) to meet health promotion guidelines and to provide protection against various chronic diseases. Both groups engaged in at least

MVPA >300min.wk^{-1} which meets the recommended physical activity guidelines for the aim of health benefits and prevention of weight gain for adults published by both the American College of Sports and Medicine (ACSM) (150-250mins.wk^{-1} of MVPA) (Donnelly *et al.*, 2009) and the Department of Health and Human Services (DHHS) (moderate >300mins.wk^{-1} or vigorous activity >150mins.wk^{-1} or an equivalent combination of MVPA) (U.S. Department of Health and Human Services, 2008). It is likely that the overweight/obese group were doing enough for health benefits and prevention of weight gain as long as their energy intake was not greater than their energy expenditure.

In 2003, the Institute of Medicine (IOM) also reported physical activity recommendations of MVPA $\geq$60mins.d^{-1} ($\geq$420mins.wk^{-1}) for adults for the purpose of weight loss and prevention of weight regain (Institute of Medicine of the National Academies, 2002). Thus, overweight/obese participants may need to increase their physical activity to meet the recommended guidelines in order to reduce body weight (body fat) and the risk of chronic diseases such as cardio vascular diseases and diabetes (Panagiotakos *et al.*, 2003; Kriska *et al.*, 2003). In previous studies, a reduction in prevalence of coronary heart disease or associated risk markers has been found to be associated with a high level of physical activity (vigorous-intensity) (Epstein *et al.*, 1976; Morris *et al.*, 1953; Morris *et al.*, 1990; Blair *et al.*, 1999). In a review of the literature, Blair and colleagues (1999) demonstrated that active obese individuals had lower morbidity and mortality than normal weight individuals who were sedentary. Furthermore, Epstein *et al.* (1976) previously investigated the relationship between vigorous exercise, other recognized coronary risk factors, and

electrocardiographic evidence of myocardial ischaemia within a group of middle age males (509 men) of similar occupational and socioeconomic status. They found that middle-aged men who reported vigorous exercise had significantly fewer electrocardiographic abnormalities than the men who did not engage in vigorous exercise. Some of the participants studied in the second study of this thesis (about 45.5% of lean and 14.3% of overweight/obese) met an average of ≥20minutes of vigorous-intensity or more for at least 3 days per week or 2 days of vigorous activity combined with another ≥2 days of moderate-intensity physical activity (U.S. Department of Health and Human Services, 2008). On the other hand, the lean group spent significantly more time in vigorous and very vigorous activity (about 67min per week) than overweight/obese participants (only about 23min per week). It is likely that this contributes significantly to a lowered cardiovascular and metabolic disease risk marker profile in these participants as well as partly explaining the differences in adiposity.

Although overweight/obese participants were moderately active and met the guidelines for health promotion, they appear to still be at risk of some chronic diseases such as coronary heart disease and metabolic disease, thus raising the question as to whether reduction of body fat content or increased physical activity (particularly vigorous activity) plays a more important role in prevention of disease.

7.2.2 Associations between physical activity status and cardiovascular and metabolic disease risk markers in lean and overweight/obese adults

Following objective assessment of physical activity for lean and overweight/obese adults, the association between cardiovascular and metabolic disease risk markers and body composition, and the association of these risk markers with physical activity status were investigated.

7.2.2.1 Associations between body composition and cardiovascular and metabolic disease risk markers in adults

The association between cardiovascular and metabolic risk factors and obesity has been investigated previously and they have been found to be higher in overweight/obese compared to lean adults (Jakicic *et al.*, 1993; Cho *et al.*, 2009; Rokling-Andersen *et al.*, 2007). On the other hand, active obese adults have been found to have lower morbidity and mortality than sedentary normal weight adults (Blair and Brodney, 1999). As just discussed the second study of this thesis (Chapter 3) observed that the habitual physical activity of lean and overweight/obese groups was not significantly different for the most commonly reported variable (MVPA). However, it highlighted that vigorous and very vigorous activity may be a key area for further investigation by public health professionals. In support of this stance the results of the third study (Chapter 4) showed that the overweight/obese participants were more likely to be at risk of disease with most of the traditional and the newer cardiovascular and metabolic disease risk markers being higher than in lean participants. Based on the

updated guidelines of the metabolic syndrome diagnosis published by the National Cholesterol Education Programme, Adults Treatment Panel III (NCEP-ATP III) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001), five of the overweight/obese participants (>22%) were found to be at risk of cardiovascular disease as they had 3 or more of the cardiovascular risk markers whereas none of the lean participants had 3 or more of the cardiovascular risk markers. In addition, 9 of the overweight/obese participants (>40%) had 6 or more of the cardiovascular and metabolic disease risk markers whereas only 1 of the lean participants (~3%) had 6 or more of these risk markers, indicating clear differences in potential for future cardiovascular or metabolic disease. Parallel with previous evidence (Brown *et al.*, 2007; Cho *et al.*, 2009), the data obtained in the third study of this thesis therefore confirms that overweight and obese adults have elevated blood lipids (total cholesterol, VLDL and LDL), higher fasting insulin and elevated blood pressure which may all indicate early stages of the development of cardiovascular and metabolic disease (Morris *et al.*, 1953; Epstein *et al.*, 1976; Cho *et al.*, 2009; Sung *et al.*, 2007). Moreover, the blood data in the third study showed that overweight/obese participants have elevated concentrations of some of the newer cardiovascular and metabolic disease risk markers comparing to lean participants. They have relatively higher plasma leptin and high sensitivity C-reactive protein (hsCRP). Plasma leptin has been found to be elevated in obese adults (Maffei *et al.*,1995; Considine *et al.*, 1996), and associated with hsCRP as a blood marker of inflammation which is a predictor for coronary heart disease (CHD) (Shamsuzzaman *et al.*, 2004; Maachi *et al.*, 2004; Ridker *et al.*, 2001). Ridker *et al.*, (2001) suggested that elevated hsCRP

with a high ratio of total cholesterol:high density lipoprotein cholesterol (TC:HDL) represents a very high cardiovascular risk marker for adults. The overweight/obese group in the third study has significantly higher hsCRP (2.64+0.42ug/ml) and high TC:HDL-C compared to the lean group. These results are probably due to high body fat and low time spent in vigorous and very vigorous activity compared to the lean group as no other differences in activity intensity were noted. Accordingly, overweight/obese participants are probably at risk of future cardiovascular and metabolic disease more than the lean participants despite the fact that they were exceeding the minimal activity guidelines suggested by many organizations. Moreover, elevated hsCRP concentration has been found to be associated with insulin resistance (Lemieux *et al.*, 2001). This is what has been found in the overweight/obese group as they had a high level of fasting insulin as well as high value of HOMA-IR. Therefore, obesity and less time in vigorous physical activity may play a major role in elevating some of the important and newer cardiovascular and metabolic disease risk markers such as hsCRP which is considered as an independent cardiovascular and metabolic risk marker. Thus, the third study reconfirms that obesity may contribute to development of cardiovascular and metabolic diseases especially when compounded with less time spent in vigorous and very vigorous physical activity.

7.2.2.2 Associations between physical activity status and cardiovascular and metabolic disease risk markers in adults

The association between the physical activity status (low active vs. high active) on some of the traditional and newer cardiovascular and metabolic risk markers was also investigated in the third study (Chapter 4). Physical activity status was found as an important factor that plays a role in number of the cardiovascular and metabolic diseases risk markers. Blair and colleagues (1999) concluded in their review that physical inactivity and low cardiorespiratory fitness are as important as overweight and obesity as mortality predictors.

Results of the third study showed that the association between physical activity and some of the traditional cardiovascular and metabolic disease risk markers was absent on many occasions and was not as strong as the link between risk markers and adiposity. In addition, there were no significant differences between the high active group and low active group in total cholesterol, VLDL, LDL, HDL or triglyceride and all values were within the normal range except the observed elevated LDL in the low active group. Similar results were found for fasting insulin, glucose and HOMA-IR as there were no significant differences between groups and all were within normal values. Although blood pressure measurements were within normal ranges in both groups, high physical activity showed healthier blood pressure status. These results concur with other findings that examined the effect of short-term exercise training on some the traditional cardiovascular and metabolic disease risk markers (Williams, 2001; Polak *et al.*, 2006). Moreover, the n6/n3 ratio was inversely correlated with vigorous and very vigorous physical activity intensity. This means that physical

activity at high intensity levels may, positively, influence fatty acid profile. Thus, exercise intensity appears to play a role in the positive changes in blood lipids and fatty acids concentrations which has been found in some other studies (Kondo *et al.*, 2006).

Adiponectin and leptin, as newer cardiovascular and metabolic risk markers have been found to be affected by physical activity and exercise training (Blüher *et al.*, 2006; Monzillo *et al.*, 2003; Wannamethee *et al.*, 2007). However, in the third study, there was no significant difference detected in adiponectin concentrations between low versus high physical activity groups. This outcome agrees with one previous study (Polak *et al.*, 2006). The influence of the exercise training observed in some studies was in obese but not in lean adults (Kondo *et al.*, 2006). Similarly, duration and intensity of physical activity may play a role in the plasma leptin concentrations (Perusse *et al.*, 1997; Elias *et al.*, 2000) which is agreed with the findings in the third study as the low active group had significantly higher plasma leptin than the high active group. However, it is not clear whether the effect of physical activity in decreasing plasma leptin concentrations mainly occurs due to weight reduction (Christensen *et al.*, 1998).

Although some studies have indicated that plasma leptin was affected by exercise training, independent of weight reduction, there were no differences between high versus low physical activity groups in plasma leptin in the present thesis when only lean participants in each group were compared. In general, high physical activity (MVPA >300mins/w) may influence the plasma leptin and adiponectin concentrations positively especially if there is a reduction in body fat. However, it is not fully clear whether or not low physically active lifestyles

(MVPA <150mins/w) is enough to influence plasma leptin concentrations positively especially in apparently lean healthy adults. Further studies are needed to investigate the contribution of physical activity status to any positive changes in plasma adiponectin and leptin concentrations in apparently healthy people, with a focus on the role that vigorous activity and adiposity may have on these markers.

In a previous study, C-reactive protein (CRP) as an independent predictor of cardiovascular disease risk was found to be affected by high intensity exercise training even in lean adults with normal concentrations (Andersson *et al.*, 2010). The high active group in the third study of this thesis showed significantly lower hsCRP concentrations than the low active group. However, the differences found between high active versus low active groups were most likely due to the number of the overweight/obese in the low active group.

When only lean participants were compared (lean high vs. lean low active groups) the results showed that although the lean high active group spent significantly more time in vigorous and very vigorous activity than the lean low active group, there was no difference between groups in hsCRP. This outcome provides some confirmation that adiposity per se and not physical activity status is a key driver for hsCRP. Moreover, there were strong correlations between hsCRP and body weight, BMI and BF% as well as with leptin but not with any of physical activity levels. Similar to adiponectin and leptin, low physical activity (about 141mins/w) might be enough for the apparently healthy lean adults to maintain hsCRP concentrations at the normal level but this does not appear to be the case for overweight/obese adults.

Plasma IL-6 and TNF- α concentrations have been investigated in adults in relation to physical exercise and exercise training does not appear to have an influence on IL-6 and TNF- α (Andersson *et al.*, 2010; Fischer *et al.*, 2004; Polak *et al.*, 2006). The third study in this thesis has found similar results. Although, both groups had normal values of plasma IL-6 and TNF- α, the high active group had lower values (Morabito *et al.*, 2007). As mentioned previously, the high active group spent more time in vigorous and very vigorous physical activity than the low active group, but circulating IL-6 and TNF- α were not significantly different. The influence of physical activity status on plasma IL-6 and TNF- α are still unclear and the effect of physical activity status seems to be not independent of other factors such as weight reduction (Monzillo *et al.*, 2003; Sloan *et al.*, 2007). Plasma IL-6 and TNF- α therefore appear to not be acute markers for predicting cardiovascular and metabolic disease in apparently healthy adults with low physical activity status. More investigations are needed in order to fully understand the role of physical activity status on circulating IL-6 and TNF-α concentration.

7.3 Health benefits of physical activity status in adolescents

Physical activity in early age such as adolescence may help to improve health in adulthood and may reduce chronic disease such as cardiovascular and metabolic diseases (Hallal *et al.*, 2006). The World Health Organization in Europe have produced physical activity guidelines for children and adolescents for the purpose of health and wellbeing including MVPA of one hour or more on

at least five days per week (Currie *et al.*, 2004). In order to evaluate the health benefits of physical activity on adolescents, the fourth (chapter 5) and the fifth (chapter 6) studies in this thesis were designed and conducted on apparently healthy Scottish adolescents (12-17yr). Physical activity was objectively assessed in the adolescents, and data was compared to other data from similar studies. Following physical activity assessment, the association between physical activity status and cardiovascular and metabolic disease risk markers was investigated.

7.3.1 Physical activity assessment in adolescents using objective measures

In the fourth study (Chapter 5), physical activity status was objectively assessed using objective measurement with the most appropriate methods for assessing physical activity in children and adolescents (as obtained from data reported in Chapter 2). The results showed that male adolescents were significantly more active than females. However, both male and female adolescents did not meet the recommended physical activity guidelines (Currie *et al.*, 2004). Habitual physical activity assessment in adolescents is a challenge because even using some of the most valid and reliable measures or methods can lead to different results which may mislead the policy makers. Therefore, the priority is to develop and establish the appropriate measures and methods for accurate physical activity assessment, especially if this assessment of physical activity is for the purpose of health promotion and disease prevention. When both

Puyau's cut-points and Freedson's cut-points were applied on the data of the Scottish adolescents the results were really misleading because Freedson's cut-points are not as appropriate as Puyau's for application to adolescents' free-living activity (Puyau *et al.*, 2002; Freedson *et al.*, 1997) but some important large scale studies still do not appear to acknowledge this (Rizzo *et al.*, 2008). Regardless of the measures and methods used for physical activity assessment in these studies, it seems that males tend to be more active than females including Scottish, European (Denmark, Portugal, Estonia and Norway) and American adolescents (Jago *et al.*, 2005; Riddoch *et al.*, 2004). When physical activity was assessed using the same instrument and method; Scottish adolescents spent significantly more time in MVPA than American adolescents in each gender separately but both failed to meet healthy living guidelines (Jago *et al.*, 2005).

7.3.2 Association between physical activity status and cardiovascular and metabolic disease risk markers in adolescents.

Data from the fifth study (chapter 6) indicated that all adolescents were apparently healthy lean adolescents. The mean of MVPA of all adolescents (n=20) was about 210 min.wk^{-1} which is less than the guidelines average ($\geq$300min.wk^{-1}) (Currie *et al.*, 2004). On the other hand, there were significant differences between active (n=9) versus low active (n=11) adolescents in counts per minute per day (CPM.d^{-1}), MVPA, and in vigorous and very vigorous activity as well as in the predicted VO_{2max}.

Cardiovascular and metabolic disease risk markers were not significantly different between groups except for fasting glucose and HOMA-IR. In addition, there was a strong association between active time, MVPA, and vigorous and very vigorous time with fasting glucose, insulin and HOMA-IR which suggests there may be initial signs of progression to metabolic disease already present in less active adolescents. Although active adolescents showed healthier levels of most of the cardiovascular and metabolic disease risk markers, both groups still recorded values within the normal range of these traditional cardiovascular and metabolic disease risk markers based on published average ranges (Medina-Urrutia *et al.*, 2008; Huang *et al.*, 2007; Andersen *et al.*, 2003; Steinberger *et al.*, 2009; Andersen *et al.*, 2006).

In a systematic review, Hallal *et al.* (2006) concluded that physical activity status in adolescents had no consistent effect on blood lipid concentrations, glucose or blood pressure. Even at low level of physical activity, these blood risk markers were not affected significantly comparing to fairly active adolescents. However, more studies may be needed to clarify whether high physical activity status can influence these risk markers compared to low active or sedentary adolescents. The balance of omega 3 to omega 6 in highly unsaturated fatty acids (HUFA) has been investigated as an inflammation marker and as a predictive index for cardiovascular diseases (Thorseng *et al.*, 2009; Lands, 2003).

Adolescents in the fifth study showed no significant differences between active versus low active groups in the n-6:n-3 (ARA:EPA) ratio and the percentage of n-3:total HUFA. Furthermore, both groups have acceptable values (Bell *et al.*,

2009; Ideal Omega test (no publication date). Available: http://www.idealomegatest.com). On the other hand, there was a fairly strong association between the examined fatty acid indexes and sedentary behaviour (positive with n-6/n-3 ratio and inversely with the %n-3/total HUFA) but not with any activity levels which may indicate that the low active or sedentary adolescents in the study are likely to have consumed a diet containing less healthy food rich in omega 3.

Similarly, the newer cardiovascular and metabolic risk markers (adiponectin, IL-6, TNF-α and hsCRP) were within the range of other apparently healthy adolescents in different studies (Rizzo *et al.*, 2008; Huerta, 2006; Eleftheriou *et al.*, 2008). There are few studies that have examined the influence of physical activity status using objective measurement methods on these cytokines. The results of a previous study using subjective physical activity measurement (questionnaire) indicated that there were no significant differences between adolescents with high vigorous physical activity versus those with low vigorous physical activity in all cytokines including adiponectin, IL-6 and TNF-α, except for adiponectin between groups in girls (Rubin *et al.*, 2008). Although the physical activity was objectively measured in the fifth study of this thesis, the results may be affected by the small sample size. Thus, to more fully explore these relationships the sample size would need to be increased and a wider range of physical activity status between groups (e.g. very active vs. low active or sedentary adolescent groups) should be considered. The second explanation for the absence of the effect of physical activity status on the examined cytokines is the body composition, as all adolescents who volunteered to

participate in the study were lean and quite active despite not meeting the physical activity guidelines. In the third study (chapter 4), the association between body adiposity and the cardiovascular and metabolic risk markers in adults were greater than physical activity. More investigation may be needed with considering sample size and a wide range of physical activity to make these comparisons in adolescent groups.

7.4 Conclusion

The new generations of Actigraph, ActivPAL and SenseWear PRO_2 are reliable devices in assessing activity EE during light to moderate locomotor activity such as walking on the flat and on a 5% incline in lean adolescents, and lean and overweight/obese adults. However, none of these devices and the methods or programme versions that were selected and applied in the first study was able to estimate activity EE accurately during walking on a treadmill. Based on the data obtained and previous evidence, the Actigraph was considered the most appropriate device for assessing the habitual physical activity due to its ability to discriminate between physical activity intensities.

Based on the results of the second and third studies, both lean and overweight/obese adults met the recommended physical activity guidelines for adults for the purpose of health and wellbeing. However, given the association between vigorous activity and adiposity, and the association between adiposity and risk markers of disease overweight/obese adults should be advised to spend more time in MVPA and in particular more vigorous activity to reduce

body fat and increase cardiorespiratory fitness in order to reduce their chances of developing cardiovascular and metabolic disease.

Habitual physical activity of the Scottish adolescents in the fifth and sixth chapters was below the recommended guidelines. When the method of physical activity assessment was adjusted the Scottish adolescents were similar to the adolescents in other European countries and were observed to be more active than adolescents in some of the developed countries such as American adolescents (Texas State). Even in the case of lean adolescents who have a low physical activity the cardiovascular and metabolic disease risk may not be obvious at this stage, but the differences in glucose and HOMA-IR suggest that there may be early signs of metabolic disease in those who are least active.

The data generated in these studies suggests that current physical activity guidelines, and health promotion messages around walking for health, may not be targeting the key issues such as the importance of vigorous physical activity. The current data and that from previous studies suggest that vigorous activity may be important for tackling obesity and thus potentially reducing risk of disease in the population. Further studies using experimental interventions are required to fully understand and explore some of the relationships identified in this current work, and these may help to guide future policy on physical activity for health in the population.

7.5 Limitations and recommendations

The present thesis contained some limitations which should be controlled in future research studies. These limitations were as a result of the time limit to complete the present studies as a PhD thesis. The common limitations of the studies in this thesis are briefly discussed in the following sections.

7.5.1 Power calculations and sample size

Sample sizes in some of the studies (chapters 3-6) were not large enough to confidently generalise the results; especially studies in chapters 5 & 6. Moreover, the risk of self selection may affect some of the findings especially in the adolescent's studies (chapter 5 & 6).

Although the fifth study (chapter 6) provided valuable information, it is best to be described as a pilot study as the sample size was less than the sample size required to generalise the results.

Data from these studies did not include any information about socio-economic status of the participants as a factor that may play a role in some of the findings (Platat *et al.*, 2006; Nguyen *et al.*, 2010). Moreover, although there are some studies that have concluded that seasonal variations do not affect the physical activity status (Ma et al, 2006, Van et al. 1986), this should be accepted with caution as it may impact the generalisability of the current findings.

In some studies (chapters 3&4), the comparison between lean versus overweight/obese participants were at risk as lean and overweight /obese groups may provide insufficient contrast in body composition which may have

overlapped in body fat content and this may impact the generalisability of the results. Thus, a larger number and a wider variation in body fat content would be recommended in future studies to provide stronger indications of differences between lean versus overweight/obese groups.

In chapters 4 and 6, it would be more benefit if a multivariate analysis was used to examine the association between physical activity and body composition and cardio-metabolic disease risk markers. Such a statistical technique is used to examine variables across multiple dimensions (body fat, physical activity, cardio-metabolic risk factors) while taking into account the effects of all variables on the responses of interest.

In addition, there is a potential risk of multiple t-test analysis used in the first study as it may lead to type I error which may provide misleading findings especially in the validity results for both adults and adolescents.

The third and fifth studies (chapters 4 and 6) were cross-sectional studies to investigate the association between physical activity status and cardiovascular and metabolic disease risk markers. Although these studies (chapters 4 and 6) have some limitations such as low sample size, beneficial findings were provided by these studies and could at as a first step for future studies. However, longitudinal or intervention studies are recommended for further study in order to provide stronger evidence on associations between physical activity and cardiovascular and metabolic risk markers and also for generalising the findings to the whole population.

7.5.2 Methodology and terminology

The new generation of AG (GT1M) has been used in both adults and adolescents (studies 3&5). Although the cut-points that have been applied on adults (study 3) were developed by Freedson and colleagues using the older model of AG (MTI 7164), the new model (GT1M) has been found to be comparable to MTI 7164 in terms of physical activity intensity classifications during habitual physical activity assessment for adults (Kozey *et al.* 2010). However, it has been recommended that when the cut-points that were developed for MTI 7164 (Puyau et al. 2002) for children and adolescents was applied on GT1M data, a +9% correction should be made (Corder et al. 2007, King et al. 2010). Thus, caution should be taken when using GT1M with the cut-points that were developed from the older AG model (e.g. MTI 7164) for both adults and children.

Foot to foot bioelectrical impedance (BIA) method has been used in all studies (chapters 2-6) to estimate body fat and fat free mass. Although (BIA) method has been found to be a valid and reliable method for estimation of body fat and fat free mass and is easy to use, as well as being low cost compared to other methods, DEXA (dual energy X-ray absorptiometry) method, for example, would have been more accurate in measuring body fat and fat free mass. Thus, further studies are recommended to use high accurate measures such as DEXA or skin fold methods when body fat is needed to be measured precisely for both adults and adolescents.

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Appendices

Appendix- A. Required documents and ethical approvals.

Enhance disclosure application

University of Stirling research ethical approval letter

NHS (Fife and Forth Valley) research ethical approval

Support letter from Stirling Council

Appendix- B. Invitation letters and leaflets.

Invitation letter to High Schools

Invitation leaflet

News paper advertisement.

Appendix- C. Research forms and letters.

Information sheet

Consent form

Physical activity readiness questionnaire

Letter to GP

Physical measurement form

PA log

Leaflet of how to use HR monitor

HR record sheet

BMI chart for children and adolescents ($\leq$20yr)

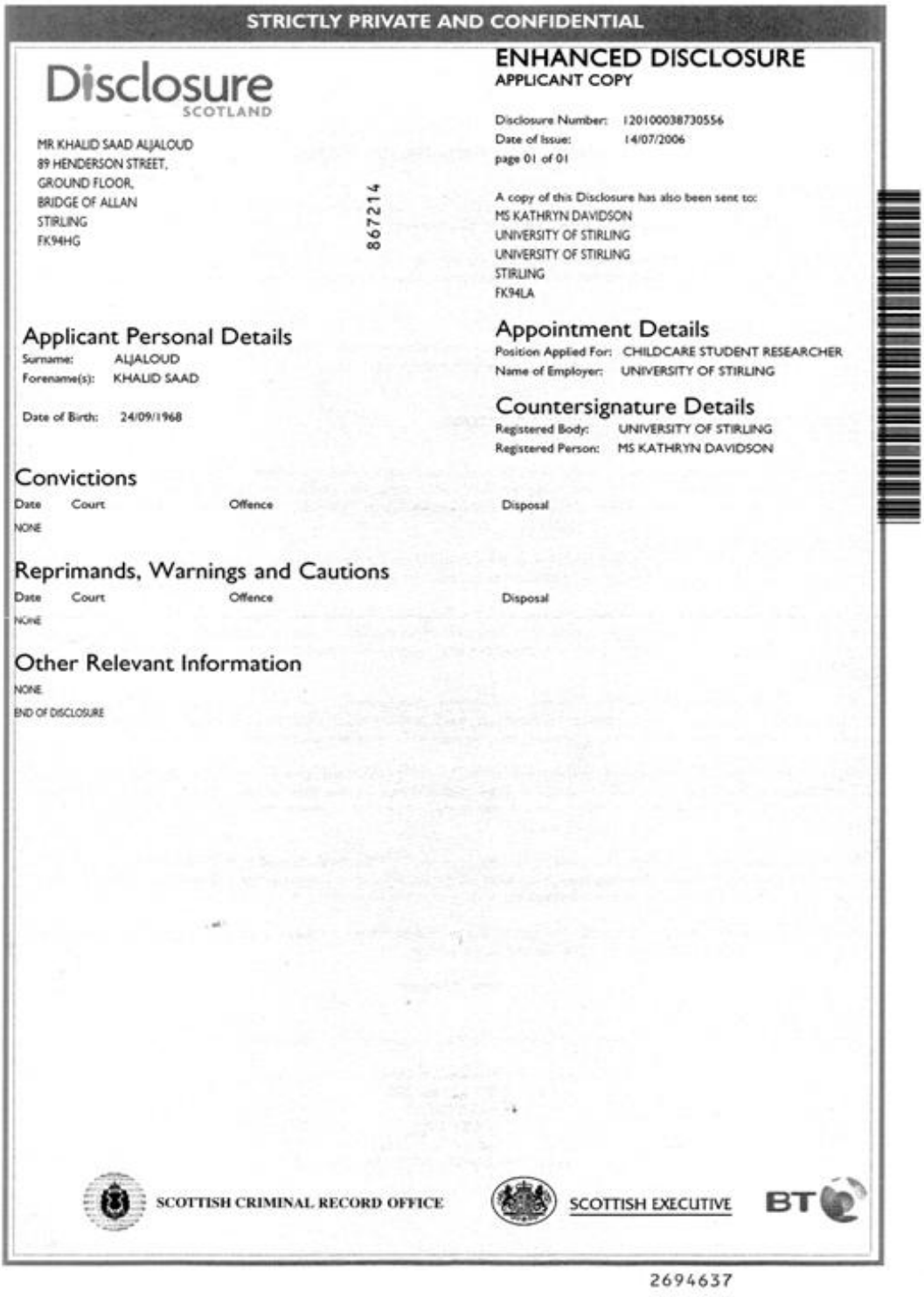
Appendix- D. Examples of data calculation.

Example of the agreement between MAHUffe software programme and manual calculation method in assessing physical activity

Example of how HR and METs are calculated

Appendix- A. Required documents and research ethical approval.

Enhance disclosure application



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Disclosure SCOTLAND

ENHANCED DISCLOSURE
APPLICANT COPY

MR KHALID SAAD ALJALOUD
89 HENDERSON STREET,
GROUND FLOOR,
BRIDGE OF ALLAN
STIRLING
FK94HG

867214

Disclosure Number: 120100038730556
Date of Issue: 14/07/2006
page 01 of 01

A copy of this Disclosure has also been sent to:
MS KATHRYN DAVIDSON
UNIVERSITY OF STIRLING
UNIVERSITY OF STIRLING
STIRLING
FK94LA

Applicant Personal Details

Surname: ALJALOUD
Forename(s): KHALID SAAD

Date of Birth: 24/09/1968

Appointment Details

Position Applied For: CHILDCARE STUDENT RESEARCHER
Name of Employer: UNIVERSITY OF STIRLING

Countersignature Details

Registered Body: UNIVERSITY OF STIRLING
Registered Person: MS KATHRYN DAVIDSON

Convictions

Date	Court	Offence	Disposal
NONE			

Reprimands, Warnings and Cautions

Date	Court	Offence	Disposal
NONE			

Other Relevant Information

NONE

END OF DISCLOSURE

SCOTTISH CRIMINAL RECORD OFFICE SCOTTISH EXECUTIVE BT

2694637

University of Stirling research ethical approval letter

UNIVERSITY OF STIRLING

DEPARTMENT OF
SPORTS STUDIES

Sport Studies Ethics Committee
Dr Angus Hunter

University of Stirling
Stirling FK9 ALA Scotland

Telephone: +44 (0) 1786 466497
Facsimilie: +44 (0) 1786 466919
E-mail: a.m.hunter1@stir.ac.uk

Mr Khalid Al-jaloud
89 Henderson Street,
Bridge of Allan,
Stirling,
FK9 4HG

6th September 06

Dear Khalid

Application no #110 "Assessment of activity energy expenditure in lean and obese school children using accelerometer devices: a validity and reliability study"

The Sport Studies Ethics Committee is pleased to announce that they are satisfied with your response to the reviewer's comments. Consequently, ethical approval has now been granted for you to proceed with your study.

I wish you the best of luck with your study.

Yours sincerely,

Dr Angus Hunter

Chair of Sport Studies Ethics Committee

NHS (Fife and Forth Valley) research ethical approval

07/S0501/50 Page 4

Fife and Forth Valley REC

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	07/S0501/50	**Issue number:**	0	**Date of issue:**	09 October 2007
Chief Investigator:	Mr Khalid Al-Jaloud				
Full title of study:	Assessment of Activity Energy Expenditure in Adults and Children Using Accelerometer Devices: A Validity And Reliability Study				

This study was given a favourable ethical opinion by Fife and Forth Valley REC on 08 October 2007. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* (1)
Mr Khalid Al-Jaloud	Full-time Ph.D. student.	University of Stirling Stirling	Fife and Forth Valley REC	09/10/2007	

Approved by the Chair on behalf of the REC:

..... (Signature of ~~Chair~~ Co-ordinator)

(delete as applicable)

..... (Name)

(1) *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.*

Support letter from Stirling Council

[redacted]

11 December 2006

Stirling Council

Children's Services

Khalid AL-Jaloud
89 Henderson Street,
Bridge of Allan
Stirlingshire
FK9 4HQ

Children's Services
Children & Families
Stirling Council
Viewforth
Stirling FK8 2ET
www.stirling.gov.uk
E-mail: ema@stirling.gov.uk
DX. ST 28
Tel. 01786 442526
Fax. 01786 442782
Director: David Cameron
E-mail: kinneyl@stirling.gov.uk
Our Ref: LK/DS
Your Ref:

Dear Khalid,

Research Request

Thank you for completing the pro-forma regarding research which you intend to carry out within the following secondary schools:

Wallace High School
Dunblane High School
Stirling High School
Bannockburn High School
St Modan's High School

I am happy to agree in principle to the research. Written permission should be obtained from individual headteachers and I note from your request that a consent form will be signed by parents.

I enclose signed pro-forma and wish you success in your project. I would be interested in your study findings and look forward to receiving a copy of the results.

Yours sincerely

Linda Kinney
Head of Learning and Development

Cc Wallace, Dunblane, Stirling, Bannockburn, St Modans High Schools

Appendix- B. Invitation letters and leaflets.

Invitation letter to High Schools

St. Modan's High School,

Barnsdale Road.

Stirling,

FK7 0PU

UNIVERSITY OF STIRLING

DEPARTMENT OF SPORTS STUDIES

Dear Headteacher, Date / / 200

I am a full-time postgraduate student from the Department of Sports Studies at the University of Stirling. I am doing a project as a part of a Ph.D. degree. The main aim of the research is to assess how much physical activity is being done by lean and obese children aged 12-17 yrs (including both male and female pupils), and to assess how good different devices are at measuring their activity. Now, following Ethics of Research Committee approval, we are in the stage of recruiting participants from secondary schools in the Stirling Council area. The first step of this stage is to identify pupils with particular body measurements (weight and height) for inclusion in our project. Therefore, we would like to pass an information sheet about our project to all pupils in your school, and their parent(s)/guardian(s), with the hope of finding some who may be willing to participate. Pupils who are willing to participate and who fall within the targeted body measurements we are examining will be invited to take part in our study.

We would appreciate the support of your school in highlighting our research and we would be willing to come in and talk to pupils at your school assembly if you thought this was appropriate. Please could you look at the attached information sheet which explains our study and reply to us by telephone, mail, email or fax if you are willing for us to come in and circulate invitation letters to your pupils. Please do not hesitate to ask us if there is anything that is not clear or if you would like more information.

Best Regards,

Principal researcher: Khalid Al-Jaloud

Tel: 01786 466476

Fax: 01786 466477

Mobile, 07775766408

e-mail: k.s.al-jaloud@stir.ac.uk

Project supervisor: Dr. Stuart Galloway

Tel: 01786 466494

Fax: 01786 466477

E-mail: sdrg1@stir.ac.uk

Invitation leaflet (Adults)

UNIVERSITY OF STIRLING

DEPARTMENT OF SPORTS STUDIES

Are you concerned about: Your weight & activity habits ?

Well, We are working on a study that measures how much physical activity is being done by healthy people. **Now**, we are in the stage of recruiting **female** participants aged 18-55yrs, with BMI * ≥ 25. Our study will help to make recommendations for participants to achieve their healthy living targets including recommended levels of physical activity and calories they expend per week and more about their healthy lifestyle. £ 25

You will be compensated for your time.

For further Information, please contact ksa1@stir.ac.uk Or Text: 07775766408

Khalid Aljaloud, Sports Studies department.

01786 466476, ksa1@stir.ac.uk

Invitation leaflet (Adolescents)

Are you interested to know

How many calories you expend per week ?

How much physical activity you are doing per week ?

Where are you in relation to the recommended guidelines ?

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Well, **we are working on a study that measures how much physical activity is being done by healthy people aged 12-17yrs, including both males and females. We are inviting you to participate in this study. Our study will help to make recommendations for you to achieve your healthy living targets including recommended levels of physical activity and calories you expend per week and more about your healthy lifestyle.**

Participants will be compensated for their time.

If you are interested to take part or find out more inf £ 25

please Contact us at any time. ksa1@stir.ac.uk

Khalid Aljaloud, Sports Studies department, University of Stirling. Fk9 4HG

Tele: 01786 466476, Mobile: 07775766408 (text) ,

Dr. Stuart Galloway Sports Studies department, University of Stirling.

01786 466494, s.d.r.galloway@stir.ac.uk

Please discuss it with your parents

For more details send an Email to:

ksa1@stir.ac.uk

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The study has been approved by NHS Fife, Forth Valley and Tayside and the Ethics Committee in Sports Studies Department at University of Stirling.

This study is supported by Children's Services at Stirling Council.

Twenty five pounds (£25) will be paid for each participant.

No >>> Diet restriction.

No >>> Physical activity restriction.

Yes >>> Number of participants is limited.

Yes >>> the study will be done in laboratory at the University of Stirling .

Thank you for reading this invitation and considering taking part in this study.

Information sheet

PARTICIPANT INFORMATION SHEET & CONSENT FORM

Habitual Physical Activity Assessment Using Objective Measuring Devices: Observations in Lean and Obese adults and children

Principal Investigator: Khalid Al-Jaloud

Other Investigator: Dr. Stuart Galloway

You are invited to take part in a research study that is being carried out in part fulfilment of a PhD doctorate degree at the University of Stirling. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part in this study.

What is the purpose of the study?

The main aim of this study is to assess how much physical activity is being done by healthy active and inactive people aged 12-50 yrs, and to assess how good different devices are at measuring activity. This will help researchers to ensure that further studies use an appropriate device for measuring energy cost when making recommendations for healthy living.

What are the qualifications of the principle researcher of this study?

Khalid Al-Jaloud has a master degree in exercise science from USA. He has sufficient experience working with children as well as adults. Since 1996, he participated in similar studies in the field of exercise physiology that published in national and international science journals. Furthermore, both researchers in this study have been cleared with an Enhanced Disclosure issued from Disclosure Scotland. It means that the researchers in this study have a clear record and they **do not have** any convictions.

Why you have been chosen?

There will be approximately 120 participants, aged from 12–50 years including both males and females. In this study, the subjects will be classified into four groups. First and second groups will include active and inactive children (~30 in each group). Third and fourth groups will include active and inactive adults (~30 in each group). Participants with these characteristics in terms of age, healthy state, and gender and activity status have been chosen to participate in this study.

Do I have to take part?

It is up to you to decide whether or not you wish to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you take part, you are still free to withdraw at any time and without giving any reason. A decision to withdraw at any time, or a decision not to take part, will not affect the legal rights you receive.

What do you have to do?

This study will involve laboratory based and free living assessment of movement sensing devices in comparison to a standard laboratory based assessment of energy cost during walking exercise on flat and on an incline (at 5% gradient) positions using a treadmill. In the first visit, you will be asked to fill out the form of Physical Activity Readiness and sign at the end of the form. You will be asked to wear three types of the movement sensing devices during two laboratory visits (one on the thigh, one on the hip and one on the upper arm). Energy expenditure assessment will be measured by collecting air breathed out during rest and exercise. There will be two laboratory sessions on two different days one to two weeks apart, and each session will last approximately 90 minutes. These sessions are undertaken at the University of Stirling, Gannochy Sports Centre. Between these visits you will be asked to wear one of the movement sensing devices and a heart rate monitor, and to record your food intake and any physical activity for two periods of 6 days. On one further occasion, you will be asked to attend the laboratory in the morning having not eaten breakfast for a blood sample to be taken (amount of 10-15ml= about 2-3 teaspoon). All participants will be compensated for their time up to a total of £25 per participant for all visits.

What are the possible disadvantages and risks of taking part?

We do not anticipate any disadvantage to taking part other than you will have to commit your time to the laboratory sessions and to recording all of the required data during the 12 days of free living activity monitoring. You may also experience some discomfort during blood sampling and possibly receive a small bruise from the blood sampling procedure but we will endeavour to keep these side effects to a minimum with blood samples being taken by a specialist trained person.

What are the possible benefits of taking part?

We hope that the output of this study will help for assessment of activity levels in children and adults in terms of tracking energy cost and that this will give you some important information about improving lifestyle and long term health. The information we get from this study may help us to more fully understand the best methods for promoting physical activity in people of your age.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. If this happens, the researcher will tell you about it. If you decide to withdraw from the study, that is fine. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information the researcher might consider it to be in your best interests to withdraw from the study and you will be notified. With your permission (both), we will inform your child's GP (family doctor) about his/her participation in this study if any unusual or surprising observations are made.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal complaints mechanisms should be available to you by contacting the University Of Stirling Ethics Of Research Committee.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be anonymised using a special code number that is stored separately on a password-protected computer file. The data about you will be for the study purposes only and will only be known to members of the research. All data about you will be stored strictly in the principal investigator's computer at the department of Sports Studies and retained for a period of 5 years after which it will be destroyed.

What will happen to the results of the research study?

We expect that the results of the study will help us to find the appropriate movement sensing device for further research and to make recommendations about the amount and intensity of activity for participants in each of the groups assessed.

The results of this study will be ready and available for you after 3-4 months. The finding might be published in some exercise science journals and might be presented in a national or an international conference.

Who has reviewed the study?

The Research Ethics Committee in the Sports Studies department at the University of Stirling has approved the study. Also, the Fife & Forth Valley Research Ethics Committee, which has responsibility for scrutinising proposals for medical research on humans, has examined this proposal and has raised no objections from the point of view of medical ethics.

If you need more information, or have any problems at any time during the study, please do not hesitate to contact one of the investigators.

Thank you for reading this Information Sheet and considering taking part in this study.

Contact for Further Information:

Khalid Al-Jaloud, Department of Sports Studies, University of Stirling.

01786 466476, 07775766408

k.s.al-jaloud@stir.ac.uk

Dr. Stuart Galloway, Department of Sports Studies, University of Stirling.

01786 466494

s.d.r.galloway@stir.ac.uk

Day	**What to do" schedule during the study**
First visit **(introductory session)**	You will be asked to attend the laboratory at the University Sports Centre and some measurements will be taken during rest state (sitting down on a chair), including heart rate and blood pressure. You will be asked to fill out a form of Physical Activity Readiness and sign at the end. Also, you will be asked to practice exercise on a treadmill and to wear some of the devices that will be used in the study. You will take enough time to try and practice what you will be asked to do in the sessions 2&3. All your questions will be answered so that you will be familiar with all tasks in this study.
Free-living measurement	You will be asked to complete 6 days in which your physical activity (daily body movements) will be assessed by monitoring heart rate, self-report of daily activity (by a daily log), and by wearing a movement sensing device.
Second visit **(lab assessment)**	You will be asked to attend 2-3 hours after you last ate. You will then be asked to perform exercise on a treadmill on the flat at speeds of 3km/h (slow walk), 4.5km/h (walk) and 6km/h (slow jog) respectively, then after 10 minutes of recovery you will perform exercise on a treadmill up a hill at speeds of 3km/h, 4.5km/h and 6km/h respectively. Heart rate, breathing and number of strides will be monitored.
Free-living measurement	You will be asked to complete another 6 days in which your physical activity (daily body movements) will be assessed by monitoring heart rate, self-report of daily activity (by a daily log), and by wearing a movement sensing device.
Third visit **(lab assessment)**	Visit to the laboratory 2-3 hours after eating you are asked to repeat the activity conducted on your second laboratory visit. Heart rate breathing and number of strides will be monitored.
	On one further occasion you will be asked to attend the laboratory in the morning having not eaten breakfast for a blood sample to be taken (amount of 15ml = 3 teaspoon). You can bring your breakfast with you to have it after taking your blood sample.

Consent form

FORM PART 2 - Copy (x1) to Ethics

Annexe B	**Ethics**	**Consent Form**

MODEL CONSENT FORM

CONSENT BY <u>PARENTS (LEGAL GUARDIAN) OF A CHILD</u> TO PARTICIPATE IN:

Name of Study: "Habitual Physical Activity Assessment Using Objective Measuring Devices: Observations in Lean and Obese adults and children"

Principal Investigator: Khalid Al-jaloud

Please initial box

1- I confirm that I have read and understood the participant information sheet dated 18th Aug 2007, version 2 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without any medical care or legal rights being affected. ☐

3- I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the department of Sports Studies at the University of Stirling, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4- I understand that these trials are part in a research study that is being carried out in part fulfilment of a PhD doctorate degree, which has been approved by the Sports Studies Ethics Committee, and may be of no benefit to me personally. The Sports Studies Ethics Committee may wish to inspect the data collected at any time as part of its monitoring activities. ☐

5- I agree to my/my child's General Practitioner being informed of my/my child participation in the study if any unusual or surprising observations are made. ☐

6- I agree to take part in the above study. ☐

Name of person taking consent ...../...../..200.... Signature

Name of the principal investigator ...../...../..200.... Signature

Physical activity readiness questionnaire

Physical Activity Readiness Questionnaire (PAR-Q)

Please print clearly and complete all sections in this form.

NAME: _____

Number: _____

There are potential risks in any physical activity programmeme. The PAR-Q has been developed to identify people that may be at greater risk, or who should see a doctor for advice prior to participation in a study quire walking and jogging on treadmill.

Please read the PAR-Q carefully and respond honestly.

Yes	**No**	
☐	☐	1. Has your doctor ever said you have a heart condition and recommended only medically approved physical activity?
☐	☐	2. Do you have chest pain brought on by physical activity?
☐	☐	3. Do you have asthma or difficult breathing brought on by physical activity?
☐	☐	4. Have you developed chest pain in the past month?
☐	☐	5. Do you have type II Diabetes (non-insulin dependent) or type I Diabetes (insulin dependent)?
☐	☐	6. Do you have anaemia (iron deficiency)?
☐	☐	7. Do you lose consciousness or lose your balance as a result of dizziness?
☐	☐	8. Do you have a bone or joint problem that could be aggravated by the proposed physical activity?
☐	☐	9. For this study, you will be asked to wear a nose clip and breathing by your mouth through a mouth piece. Do you suffer from claustrophobia?
☐	☐	10. (**Only for female**) are you in any stage of pregnancy?
☐	☐	11. Is your doctor currently prescribing medication for you? If so, list the medications and the reason for the medication.

Medication **Reason for Medication**

_____

Yes	No	
☐	☐	12. Do you have particular fears or worry about participating in physical activity? If yes, indicate:

_____

13. **Informed consent.**

I, the undersigned, acknowledge that there are potential risks in physical activity programmes. I assume those risks and consent to the proposed participation in this programme. I, or any person claiming through me or on my behalf, do hereby release and agree to save harmless Sports Studies Department of Stirling University, the department and their staff, employees or workers, from all claims for loss, injury or damage to persons and property while participating in this programme, or travelling to or from this programme, except when the University and/or its agents are negligent.

Parental signature: _____ **Date:** _____

NOTE: If you answered YES to any question from 1 through 12, consult your doctor before allowing you to participate in the study. Failure to do so may increase your injury/health risk of participating in the study.

Other Special Requests:

_____

Letter to the GP

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Dear General Practitioner

Your patient: …… has been invited to take part in a study investigating the validity and reliability of three types of movement sensing devices. We would like to inform you that this study involves some measurements including taking blood sample (10ml), and assessment energy cost of activity using indirect calorimetry during walking exercise on a treadmill at flat and on an incline(5% gradient). Also, body fat measurement using bioimpedance analysis will be obtained in this study. We do not anticipate and discomfort other than normally encountered through blood sampling, and moderate exercise.

If you have any concerns about any health related problem regarding the participation of the above named patient, please contact us via telephone, fax, or email.

Many thanks

Researchers

Principle researcher: Khalid AL-jaloud [01786-466476, [k.s.al-jaloud@stir.ac.uk]

Other researcher: Dr. Stuart Galloway [01786-466494, [s.d.r.galloway@stir.ac.uk]

Fax No. : 01786-466477

Letter to the GP

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Letter to the GP

Dear General Practitioner

Your patient: ..... has been invited to take part in a study investigating free living activity and exercise test in the lab involving walking on treadmill. We would like to inform you that during his/her participation, some unusual/surprised results have been observed. These unusual/surprised observations are:

……

……

……

If you need further information about your patient above named, please contact us via telephone, fax, or email.

Many thanks

Principle researcher

Khalid AL-jaloud

Tel: 01786-466476]

Fax: 01786-466477

k.s.al-jaloud@stir.ac.uk

Physical measurement form

Physical Measurement form

Dear Participant

If you would like to take part in the study, please, complete the information below. As indicated in the information sheet, ***all information will be secure and will be used only for the purpose of the study***.

Name: …….. **Code No.:**

School Name (if any): ……..

Birthday: / / 19 **Age:** **Gender: Male** ☐ **Female** ☐

Name of parent(s), guardian: …….

Address: …….

City, Town: ……..

Post Code: ……..

Contact details:

Telephone: day time: …… / **Evening time:** …….

Email: **@**

Weight: (**Kg.**): **Height (Cm.)**: **BMI:**

Resting HR: **Blood Pressure (mm Hg):** ..... **VO_{2Max}:**

Body fat **Body-fat percentile** **Fat-free mass**

Start Date: / / 200 **Time:**	**Stop Date: / / 200** **Time:**	**Session: 1. 2. 3.**
ActiGraph S.No.:	**HR Monitor Polar S.No. :**	**ActivPal S.No.:**

Diary of physical activities assessment (youth)

Please, fill in physical activities with **moderate intensity and above** you took part in throughout the day including **type**, **duration** and **intensity** of these activities.

Only include types of physical activity with **moderate and above** intensity. **Do not include** any physical activity with **low intensity** such as walking inside the house, cooking, drawing, reading, watching TV…etc
Low intensity activities: your heart rate and breathing **are no different** from what they are when sitting or standing. **Moderate intensity** activities (moderate physical effort): your heart rate and breathing **somewhat faster than normal**, similar effort as a brisk walk (e.g. washing windows, sweeping, mopping, vacuuming …etc). **Vigorous intensity** activities (hard physical effort): your heart rate and breathing **much faster than normal** and you have to **breathe deeper**. Also, you probably sweat. (e.g. walking briskly uphill, aerobics, digging, jogging). **Very vigorous intensity** activities (very hard physical effort): your heart rate and breathing **are very fast** and usually, **you sweat** when you reach this level of physical effort (e.g. running, weight-lifting, mountaineering …).

Here is an example of how to fill in the diary of your physical activities.

Day: Wednesday, Date: 12 / 05 / 2008

Diary of physical activities assessment

Time	**Type of physical activity**	**Duration** How many minutes did you spend in this type of activity?	**Intensity**	**Note**
Morning	(e. g. washing your car)	(e. g. roughly 10 min.)	(e. g. Moderate)	
Afternoon	---	---	---	No physical activity
Evening	(e. g. playing football match) hard game (e. g. Dancing)	(e. g. 60min) (e. g. 15 min)	(e. g. Vigorous to Very Vigorous) (e. g. Moderate)	10min rest between 1st and 2nd half.

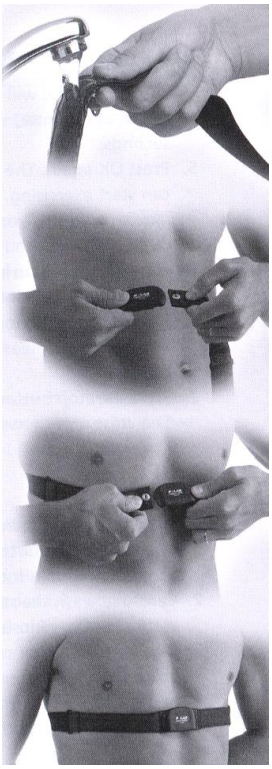
Day: **Wednesday,** **Date:** **12 / 05 / 2008**

Diary of physical activities assessment

Time	**Type of physical activity**	**Duration** How many minutes did you spend in this type of activity?	**Intensity**	**Note**
Morning				
Afternoon				
Evening				

Leaflet of how to use HR monitor

Measuring heart rate using *POLAR* HR Monitor



HR monitor – step by step instructions

Step 1. When you get up in the morning wet the front part of the strap with warm water and put the strap round your chest as shown in the diagram. Ensure that the strap is in good contact with your skin (you may need to tighten the strap a bit to achieve this).

Step 2. Put the watch on your wrist and hold it up to your chest and press the big red button once. You should see some lines appearing that circle clockwise as the watch searches for a signal. When a heart rate appears (you will see the heart shape on the bottom left registering a signal and a number in the middle at the bottom will appear – this is your heart rate in beats per minute). Once this appears press the red button once again and the timer will start.

Step 3. If taking off the monitor for a brief time to shower or swim etc. then press the stop button once (see diagram, bottom left hand side of watch) and leave the monitor lying. When you put it back on make sure you wet the front part of the strap again, put it on and press the red button once (your heart rate should appear and timer should continue from where you stopped).

Step 4. When taking the monitor off at night press the stop button remove the strap from your chest and leave until morning. In the morning your monitor will have returned to time of day mode and you should go back to step 1 to record your next days heart rate.

Troubleshooting

Monitor says my heart rate is 00.

If you notice that your heart rate monitor is reading 00 but the timer is still running try wetting the underside of the front part of the strap by wetting your fingers and running them along the strap either side of the central fitting point. Then hold the watch up close to your chest and see if the signal is restored. Once you have a signal the watch will begin to record your heart rate again and a number will appear instead of 00.

Monitor says 'Sensor'

If the monitor says 'Sensor' (usually after only wearing for a minute or two) press the red button again once.

Monitor says 'Memory full'

If this happens contact Khalid as soon as possible and he will download it and return it to you straight away.

Step 3 above doesn't work

If step 3 doesn't work when you take off your monitor then check that the timer is stopped. Once the timer is stopped press the top right hand button on the watch until you see the time of day as the main display. Once you have this follow the procedure in step 1 and note down what time this occurred in your activity log.

How to detach the connector from the strap:

1- Apply pressure with your thumb and forefinger and turn your hand as indicated in the picture.
2- Carefully wash the strap with a mild soap and water solution. Rinse it with pure water.
3- Dry the transmitter carefully with a soft towel.
4- Store the transmitter in a clean and dry place. Dirt impairs the elasticity and functioning of the transmitter. Sweat and moisture can keep the electrodes wet and the transmitter activated, which shortens battery life.

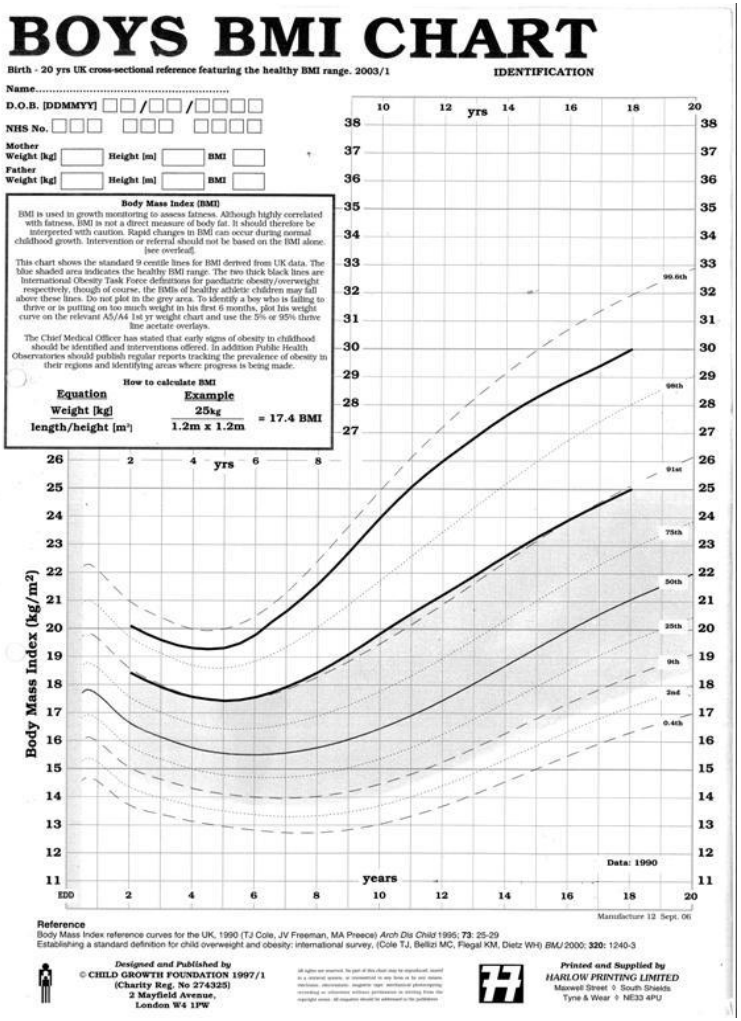
HR record sheet

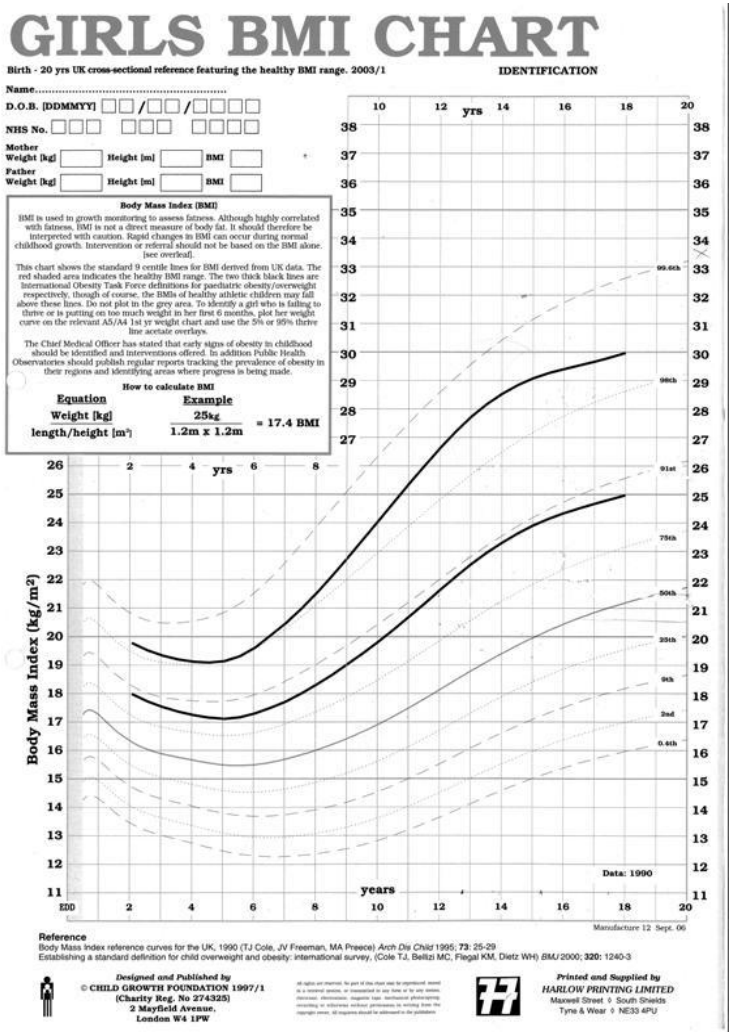
Day : **Date: / / 200**

Name:	**Code No.:**	**Rest Start: :** **Exercise Start: :**
Resting Heart Rate: bpm	**BMI:**	**Exercise Stop: :**
ActiGraph S.N:	**HR Mon. Polar S.N :**	**ActivPal S.N:**

HR / Speed	1st min	2nd min	3rd min	4th min	5th min	6th min	7th min	8th min	9th min	10th min
Flat 3 k/h										
Flat 4.5 k/h										
Flat 6 k/h										
Recovery 10 min										
5%Up 3 k/h										
5%Up 4.5 k/h										
5%Up 6 k/h										

BMI chart for children and adolescents (≤20yr)





Appendix- D. Examples of data calculation.

Example of the agreement between MAHUFee software programme and the manual calculation method using Actigraph data.

1 day Physical activity assessment for a participant using MAHUffe software programme.

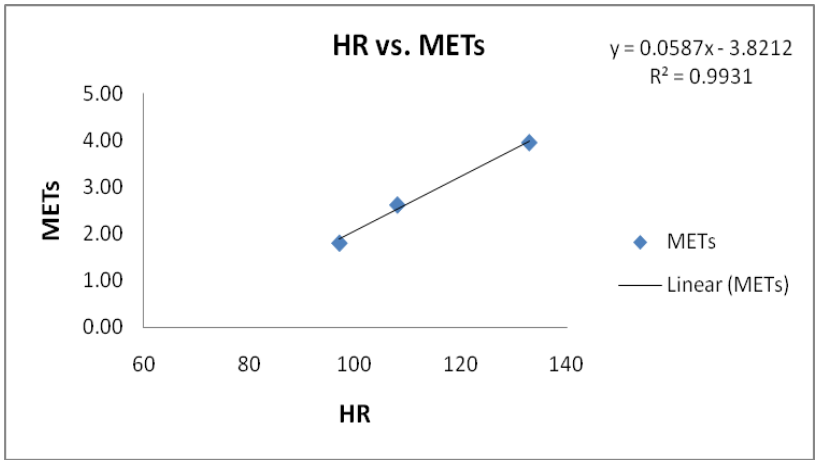
Volunteer	Epoch	Day	Sedentary	Active	Light	Moderate	Vigorous	V Vigorous	RegTime	Count	Count/Min
LAF15-3	**60**	**21-May-08**	**855**	**56**	**37**	**19**	**0**	**0**	**781**	**208788**	**267.33**
LAF15-3	60	22-May-08	1360	80	70	10	0	0	945	246556	260.91
LAF15-3	60	23-May-08	1330	110	82	28	0	0	948	330613	348.75

1 day Physical activity assessment for a participant using Manual calculation method.

Initial	
Final	
n Epochs in Hour	
n Mins in Hour	
Sum cpm in Hour	
Total Mins in Day	781
Total cpm in Day	208788
Mean cpm per Day	267
Total Hours	13.0
Times Used	
Parent Recorded Times	
Sedentary (800)	
n sedentary Mins in Hour	
% Mins in Hour	
Total sedentary Mins	706
% Total Mins in Day	90.39693
Sedentary (1100)	
n sedentary Mins in Hour	
% Mins in Hour	
Total sedentary Mins	725
% Total Mins in Day	92.82971
Light	
n Light Mins in Hour	
% Mins in Hour	
Total Light Mins	37
% Total Mins in Day	4.737516
Moderate	
n Moderate Mins in Hour	
% Mins in Hour	
Total Moderate Mins	19
% Total Mins in Day	2.432778
Vigorous	
n Vigorous Mins in Hour	
% Mins in Hour	
Total Vigorous Mins	0
% Total Mins in Day	0
Inactive	
n Active Mins in Hour	
% Mins in Hour	
Total Active Mins	725
% Total Mins in Day	92.82971
Active	
n Active Mins in Hour	
% Mins in Hour	
Total Active Mins	56
% Total Mins in Day	7.932011

Example of how free-living activity was assessed using the laboratory measured HR and METs.

Flat	HR	METs
3km/h	97	1.80
4.5km/h	108	2.62
6km/h	133	3.96



Y = 0.0587*X - 3.8212, Y= METs, X= HR

The regression equation that obtained from HR and METs for each individual participant during exercise walking on treadmill on the flat was used to assess the habitual physical activity for that participant.