Effects of the *FTO* gene and Environment on Obesity in European Children

By

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To my Mom and Dad

Abstract

Childhood obesity is considered to be one of the most serious public health problems of the 21st century. The worldwide prevalence of obesity has increased dramatically over the past three decades and while continuing to rise at a rapid rate, along with increasing levels of childhood obesity, are having a profound effect on healthcare development in many low- and middle-income countries, particularly in urban environments. Longitudinal and cross sectional studies have indicated clear associations between environment and obesity risk. In addition, childhood obesity leads to serious health conditions such as cardiovascular disease, type II diabetes and adulthood obesity. Environmental factors, however, do not seem to explain neither all of the variance in childhood obesity prevalence, nor all the variance in response to intervention studies. Although the human genome has not changed over the years, obesity levels and mortality rates have dramatically increased, thus it becomes more evident that environmental factors such as physical activity or sedentary lifestyle may have a key role in this increase of obesity prevalence. However, since the prevalence of childhood obesity is different in certain geographical areas of the world, it is important to investigate the genetic predisposition in relation to its interaction with environmental influences. Genetic studies have demonstrated a contribution of specific genetic variants to obesity in adults. Additionally, heritability studies of childhood obesity support the idea that genetic predisposition may also be a factor in determining childhood obesity or adiposity. The obesity prevalence research becomes even more complicated by gene-environment interactions, where individuals with different genotypes respond differently in certain environments and therefore it is more challenging to define the actual causes of this health problem.

The overall aim of this study was to investigate the interplay between genetic and environmental influences such as physical activity on the predisposition to childhood obesity related traits in the IDEFICS cohort. This thesis focused on European children from eight countries participating in the IDEFICS Study including Germany, Italy, Spain, Cyprus, Estonia, Sweden, Belgium and Hungary aged two to ten. The main objective was to characterize the relative contributions of individual genes, environmental factors and gene-environment interactions to this risk. In doing so, this investigation also allowed comparisons between the different age groups and countries and also possible differences between the two sexes. These findings will add to the existing efforts aimed at finding appropriate treatments and effective preventative intervention programs around Europe. In order to explore how environment and genes interact and whether genes can modulate the development of obesity in children of this European population, a detailed characterization of body composition, physical activity patterns, socio-economic, and genetic factors was performed.

The main findings from this thesis were that: (a) age is an important factor when studying childhood obesity as body composition changes in a significant way with age, in both boys and girls. These findings also highlight the fact that various environmental and lifestyle effects

related to childhood obesity, such as physical activity (PA), differ between the two sexes and among age groups; (b) physical activity and sedentary behaviours may influence obesity related phenotypes in children of European origin. These associations persist after adjustment for a comprehensive range of potential confounding factors; (c) the Fat Mass Obesity-associated (*FTO*) gene influences obesity related phenotypes in children of European origin. These associations persist after adjustment for a comprehensive range of potential confounding factors; (d) although there was no Gene*PA interaction, physical activity or inactivity seems to play a role in modulating the genetic predisposition to obesity in children. The findings of this study demonstrate that there was a trend of decreased obesity risk phenotypes in children that were more physically active overall. This observation has important public health value, as the data of this thesis indicate that being physically active may have a protective role in the genetic predisposition to obesity induced by variation in the *FTO* gene. Further studies into the mechanisms underpinning this effect are needed in order to more effectively develop accurate design, as well as implementation strategies to reduce childhood obesity and for advancing the basic understanding of the mechanisms behind human obesity and its relationship with genetics.

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Author's declaration

Unless otherwise indicated by the acknowledgment or reference to published literature, the present work in this thesis is the author's own and has not been submitted for a degree at another institution.

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Definitions

ABI	Applied Biosystems
AVG_CPM	Average Counts per Minute
BIA	Bioelectric Impedance
BMI	Body Mass Index
BMR	Basal Metabolic Rate
CHD	Coronary Heart Disease
CI	Confidence Interval
dbSNP	Single Nucleotide Polymorphism database
DNA	Deoxyribonucleic Acid
EE	Energy Expenditure
EI	Energy Intake
FTO	Fat Mass and Obesity-associated gene
GLM	General Linear Model
GWAS	Genome Wide association Study/studies
HWE	Hardy-Weinberg Equilibrium
IDEFICS	Identification and prevention of Dietary- and lifestyle-induced health Effects In Children and InfantS
IDF	International Diabetes Federation
Kg	Kilogram(s)
miRNA	MicroRNA
MVPA	Moderate-to-Vigorous Physical Activity
NAFLD	Nonalcoholic Fatty Liver Disease
OR	Odd Ratio
PA	Physical Activity
PCR	Polymerase Chain Reaction
SD	Standard Deviation
SES	Socio-economic Status
SNP	Single Nucleotide Polymorphism

1 Introduction

This chapter aims to provide the relevant scientific background for each chapter and establish the theoretical basis for this study. This chapter begins with an overview of childhood obesity, the public health impact worldwide and also with a specific overview on population of European children. Furthermore, this is a more detailed examination of the potential contributing factors in obesity which will help us to understand better the lifestyle aspect as well as genetic components of this epidemic condition. Finally, the potential role of genes and environment interaction in the development of obesity is explored and explained.

1.1 Obesity epidemic: a worldwide problem

Obesity can be defined as an excess accumulation of body fat that leads to an increased risk of morbidity and/or premature mortality (Reilly 2005). Obesity is commonly defined in terms of body mass index (BMI>30 in adults, and over the 95th percentile in children) and is calculated as weight divided by height in meters squared. In children, a BMI that is less than the 5th percentile is considered underweight and above the 95th percentile is considered obese; however, individuals under 20 years old with a BMI between the 85th and 95th percentile are considered to be overweight (Barlow 2007). These age and sex specific percentiles were created to assess childhood obesity due to the fact that the amount of body fat changes with age and also differs by sex. In addition, these percentiles were prepared considering these differences and allow for a more accurate BMI estimate in young children. However, when using these percentile cut-offs in children it means that the 'prevalence' is already set to 5%, and therefore an increase in prevalence is then interpreted as the study of the change in BMI value at which the 95th percentile is found and not the actual change in prevalence. Therefore, prevalence studies must be interpreted with special care as they generally use a reference study and will focus on how much the % of children above the cut-off values of that reference study is changed over time.

The increase in the prevalence of obesity seems to have begun almost simultaneously in most high-income countries during the 1970s and 1980s (Sassi F 2009). Since then, most middle-income and many low-income countries have joined the global surge in obesity prevalence in both adults and children as Finucane et.al (2011) predicted. This prediction was calculated using estimated trends and their uncertainties of mean BMI for adults of 20 years old and older across 199 countries and territories. In addition, data from previous health examination surveys and epidemiological studies (960 country-years and 9.1 million participants) were obtained and a Bayesian hierarchical model was used to estimate mean BMI by age, country and year (WHO 2008; Finucane et al. 2011). Comparisons between BMIs determined in 1973 at the onset of the

Bogalusa Heart Study and those from 1992 to 1994, showed a significant increase of approximately 12 pounds on average per child without a significant increase in height (Baker et al. 2007). Additional data in Bogalusa 2009 study have shown that this increase has been maintained and an average increase of overweight in children by approximately 17 pounds was observed (Broyles et al. 2010). By 2008, an estimated of 1.46 billion adults globally were overweight (body-mass index [BMI] >25 kg/m²) and 502 million adults were obese (BMI >30 kg/m²) (Finucane et al. 2011). Furthermore, a remarkable estimate of 170 million children (aged <18 years) globally were classified as overweight or obese (Lobstein et al. 2004). Figure 1.1 demonstrates this estimate showing that more than 25% of all children in certain countries are overweight or obese and that the numbers of such children more than doubles since the beginning of the epidemic. This estimate was calculated using the International Obesity Taskforce cut-offs which are used for children and adolescents of 2–18 years old and were developed from a database of 97 876 boys and 94 851 girls, from birth to 25 years and from six countries (Brazil, Great Britain, Hong Kong, the Netherlands, Singapore and the USA) (Cole et al. 2000). There is limited analysis of the patterns of the obesity epidemic in the past four decades due to the absence of representative data from different countries (Wang and Lobstein 2006). However, the pattern by which obesity prevalence increases in particular populations seems predictable. It has been observed that low-income and middle-income countries, groups of high socioeconomic status in urban areas tend to be the first to have high obesity prevalence, but the burden of obesity shifts to low socioeconomic status groups and rural areas as a country's gross domestic product (GDP) increases (Monteiro et al. 2004; Mendez et al. 2005). In Brazil for example, one of the few middle-income countries with repeated cross-sectional surveys of BMI, this pattern was particularly evident for women, with obesity rates increasing rapidly in the lowest income groups (Monteiro et al. 2007).

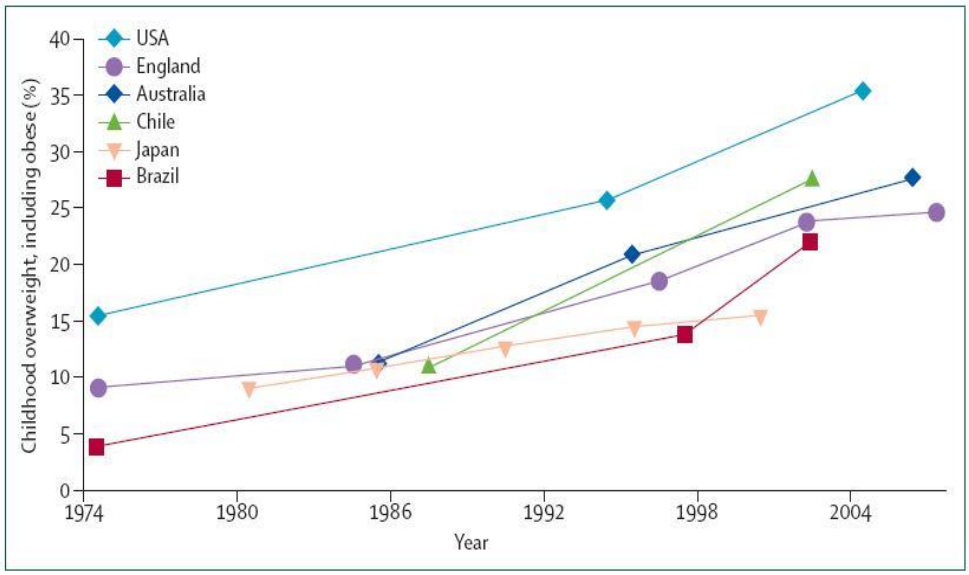

Figure 1.1 Estimates of percentage of childhood population overweight, including obese (with use of International Obesity Taskforce cut-offs) in a selection of countries (Wang and Lobstein 2006).

According to the World Health Organization (WHO), obesity accounts for 10–13% of deaths in many European countries, (WHO 2010a) indicating a necessity to investigate further all the factors causing obesity and design interventions to treat or prevent it. Furthermore, estimates for the year of 2008, showed that over 50% of both men and women were overweight and approximately 23% of women and 20% of men were obese (WHO 2010b).

A remarkable prediction that has been estimated is that by 2030, up to 58% of the world's adult population will be overweight or obese (Kelly et al. 2008). In addition, according to World Health Organization (Zinn et al. 2011), overweight and obesity are the fifth leading risks associated with global deaths. Studies show that at least 2.8 million adults die each year as a result of being overweight or obese. Furthermore, obesity is found to be highly associated with other chronic health conditions. Interestingly, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity (WHO 2011). The increase in obesity has not been confined exclusively to adults, as childhood obesity has been rapidly increasing over the years. The World Health Organization supports that in 2010; around 43 million children under five years old were overweight. According to studies, very close to 35 million overweight children are living in developing countries and 8 million in developed countries (WHO 2011).

1.1.1 Childhood Obesity in Europe

Obesity in European countries has been increasing in a similar way like in other parts of the world. Its prevalence has tripled in many countries of WHO European Region since the 1980s and the affected countries continue to rise at an alarming rate, particularly among children, as the number of overweight infants and children rose steadily from 1990 to 2008 (WHO 2010). Due to the plethora of definitions and cut-off criteria which are used to define overweight and obesity in children, intra- and inter- country comparisons of the prevalence in childhood obesity are difficult to estimate (Livingstone 2001).

Overweight prevalence in childhood and adolescence (7 and 11 years old) was compared in two nationally representative cohorts of British children born in 1946 and 1958 (Stark et al. 1981; Peckham et al. 1983). Although the prevalence of obesity among 7-9 year-olds born in 1958 was nearly twice that among those born in 1946, the difference between the two cohorts had almost disappeared by 14 and 16 years. This finding suggests that factors predisposing children to obesity may change over time, and that age seems to be an important factor when estimating obesity prevalence in children. A possible explanation for the difference in obesity prevalence at the age of 7, may be due to food rationing which influenced nutrition during 1946 and continued post-war until 1954 (Peckham et al. 1983). In addition, there is evidence that by 1954, the nutritional value of the national diet had reached a peak and remained stable (Hollingsworth 1961). As a result, up to the age of 7, the 1958 cohort may possibly have had a higher energy intake than that of 1946; however by the time each cohort had reached adolescence, there was probably very small, if any, difference in the national diet. Furthermore, mixed longitudinal data

showed that between 1972 and 1994, triceps skinfold thickness had increased by 8% and 7% in English boys and girls respectively and by 10% and 11% in Scottish boys and girls respectively (Hughes et al. 1997). Considering the above findings, these data suggest that the prevalence of obesity in British children is continuing to increase in line with the increased prevalence in British adults (Seidell 1995).

Due to the cross-sectional nature of the European data on the prevalence of childhood obesity, it is very difficult to track the development of adiposity in children and cause-and-effect relationships cannot be established. In addition, population samples are often small, and the randomness of the population groups even in large samples is uncertain (Livingstone 2000). However, the available European prevalence data, although not perfect, still show that childhood obesity is increasing throughout EU countries and the patterns in prevalence vary with time, age, sex and geographical region. Hence, in the Netherlands for example, weight-for-height indices for children remained stable from the 1950's to the 1980's (Blokstra and Kromhout 1991). However, since then, there has been an increase in the prevalence of obesity observed in Dutch adults over the same period (Seidell et al. 1995). The trend for rates of obesity showing marked increase from the 1980's has been also observed in Switzerland (Woringer V 1998) and in France (Rolland-Cachera MF 1992).

According to the overall obesity pattern, it is suggested that prevalence rates in young children are lower than in adolescents. However, data from the UK are of particular concern since they demonstrate an excess of overweight (19%) and obesity (7%) in 5-year-olds (Reilly et al. 1999). This fact highlights the need to initiate effective preventive strategies and interventions, even prior to children reaching school age.

Studies in the prevalence of obesity find inconsistent results as to the sex differences. For example, Italy (Maffeis et al. 1993), Finland (Nuutinen et al. 1991) and Austria (Elmadfa I 1993) showed the highest prevalence of obesity among boys, while other studies conducted on British (Peckham et al. 1983), Italian (Maffeis et al. 1998) and Spanish (Moreno LA 1998) sub-populations demonstrated the opposite trend. These data differences could be due to biased sampling or the considerable geographical variation in the prevalence of paediatric obesity. The highest rates of obesity are observed in southern European countries rather than in central and eastern Europe (Figure 1.2). Furthermore, countries surrounding the Mediterranean presented prevalence rates for overweight children in the range of 20–40%, while those in northern areas showed rates in the range of 10–20% (Lobstein and Frelut 2003). Lobstein et al. attribute the low prevalence in eastern and central regions to the economic recession these regions have experienced (Lobstein and Frelut 2003). However, although Lobstein et.al data on the overweight and obese children in Europe are presented in an interesting geographical way, the findings of this study should be treated with caution. The standardized BMI cut-offs were developed using data from six populations (UK, USA, the Netherlands, Brazil, Singapore and Hong Kong), which were combined to provide the reference population. This range of populations may not properly reflect the

trends and health risks within specific countries (Vignerova et al. 2001). In addition, BMI does not account for body fat and lean body mass, which may be important for children and adolescents at particular stages of growth; hence this study did not account for age specific overweight and obesity prevalence. Furthermore, other environmental parameters such as dietary intake, household income levels as well as physical activity are important when estimating obesity prevalence in a specific population of which the above data are absent.

Obesity is clearly a major public health problem in Europe. However, the size of the problem is yet unclear and hard to determine, as obesity definitions and small number of cross-sectional data are causing biases to the actual European obesity prevalence. In addition, during both preschool years and puberty, body composition undergoes marked changes as developmental processes occur. From the late 19th to mid-20th century a gradual decline in age at puberty has been reported in girls, after which this trend ceased, most likely as a result of increased stability in socio-economic conditions, nutritional status and hygiene (Parent et al. 2003). Puberty is the end-point of a complex series of developmental events, and identification of the trigger(s) of pubertal onset has drawn considerable attention. The pubertal cascade is initiated by the activation of the hypothalamic-pituitary-gonadal axis leading to the development of secondary sexual characteristics. Research efforts are presently ongoing to unravel the complex nature of the activation of this axis (Tena-Sempere 2012).The 'critical weight' hypothesis suggested by Frisch and Revelle during the early 1970's proposed a minimum weight of 48 kg or 22% body fat to allow puberty to start (Frisch and Revelle 1970; Frisch and Revelle 1971). However, recent studies have shown that leptin, produced by the white adipose tissue in proportion to body energy stores, plays an (in)-direct role on the onset of puberty (Tena-Sempere 2012). Recent secular trends in pubertal maturation seem to coincide with the increasing prevalence of overweight and obesity (Lee et al. 2001) and have raised considerable discussion as to whether early maturation is due to the obesity epidemic.

During the first decade of life, body composition is changing in absolute and relative proportions of water, lipid, protein and mineral mass; at birth, for example, approximately 80% of lean tissue is water, declining to approximately 75% at the end of the 1st decade (Fomon et al. 1982). Furthermore, there is evidence that adipocyte number is a major determinant of fat mass in adults (Spalding et al. 2008). Spalding and al. assessed the total adipocyte number in 687 adult individuals and combined this data with previously reported results for children and adolescents. (Spalding et al. 2008). It was found that even though total adipocyte number increased in childhood and adolescence, this number leveled off and remained constant in adulthood in both lean and obese individuals. This demonstrated that the difference in adipocyte number between lean and obese individuals is established during childhood and the total number of adipocytes for each weight category stays constant during adulthood. It is therefore established that adipocyte number for each BMI category is a stable cell population during adulthood and this is important if we take into account that most obese adults have been obese since childhood, and less than 10% of children with normal weight are going to develop adult obesity. In addition, future research should aim to better understand the causes of the observed differences in prevalence across

Europe, as well as environmental and genetic factors that may influence the obesity problem in children.

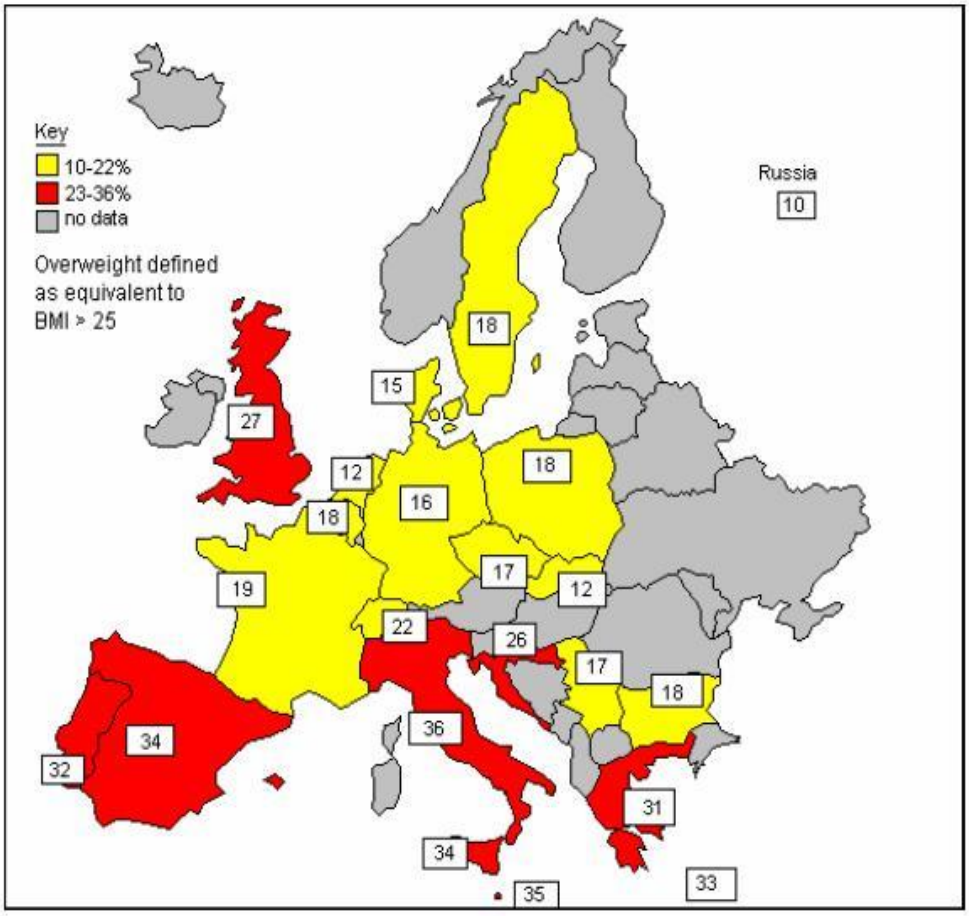

Figure 1.2 Prevalence of overweight in children aged 7-11 years across Europe (Lobstein and Frelut 2003) .

1.2 BMI as obesity definition measure in children

1.2.1 Body Mass Index

Obesity can be defined as the excess accumulation of body fat, which puts a person at increased risk of morbidity and premature death (WHO 1995; Reilly 2005). However, giving precise definitions to obesity can be challenging, as there are no clearly defined cut points available where body fat is considered excessive. For this reason, in most scientific epidemiological studies, relative measures of weight are used as a proxy of fat (Ogden et al. 2007). Body Mass Index (BMI) is the most commonly used obesity definition and is calculated by dividing weight in kilograms by height in meters squared (kg/m^2). World Health Organization (WHO) developed a classification of BMI for international use, principally based on its association with mortality; with overweight defined as 25 to 29.9 kg/m2, obese as 30-39kg/m2 and morbidly obese as 40

kg/m2 and above (WHO 1995). The detailed classifications of adult underweight, overweight and obesity according to WHO cut points is presented in Table 1.1.

Table 1-1 The International Classification of adult underweight, overweight and obesity according to BMI.

Classification	BMI(kg/m^2)	
	Principal cut-off points	Additional cut-off points
Underweight	<18.50	<18.50
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	18.50 - 24.99	18.50 - 22.99
		23.00 - 24.99
Overweight	≥25.00	≥25.00
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	≥30.00	≥30.00
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

Source: Adapted from WHO, 1995, WHO, 2000 and WHO 2004.

Body mass index measure has the advantage of being very easy to use and is a non-invasive method that requires only the accurate collection of height and weight, thus making it the most applicable method in epidemiological field work and clinical setting studies. However, BMI measure also has limitations and most importantly it does not provide an accurate estimate of body fat. In addition, BMI and body fat relationship becomes more complicated due to several factors. Firstly, BMI measure is not a very efficient measure of obesity in individuals with high muscle mass. Due to the fact that muscle mass is heavier than fat mass, BMI measure may cause overestimation of body fat composition (Prentice and Jebb 2001). In addition, body fat percentage varies with ethnicity. For example, Caucasian populations have higher fat percentages than African-Caribbean's (Prentice and Jebb 2001). Furthermore, since the amount of body fat is age- dependent, BMI may cause over and/or under estimations when estimating body fat of children at different age range.

1.2.2 Body Mass Index in Children

As previously mentioned, BMI as an obesity measure has certain limitations and it becomes even more complex when it is used in young children as it appears to be age and sex dependent (Cole et al. 1995). Evidence in the literature shows that there have been underestimations of the obesity prevalence in children due to the relative contributions of body fat and fat free mass to BMI changing over the years in children (Wells et al. 2002). In addition, since BMI is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2), it is correct to say that it is used to determine the fattest children in a specific population but is not an actual measure of body fat content (Lobstein et al. 2004).

Furthermore, the limitations of BMI as a measure lead to the creation of two kinds of BMI reference data for the definition of childhood obesity; the national and the international reference data. National reference data exist for the USA, Asia and several countries within Europe and was produced from height and weight data in large representative samples of children. National data can be used in the form of age and sex specific z-scores (the number of SDs away from the mean BMI), or by using percentile cut offs. For epidemiological purposes, children should be considered overweight when they are above 85^{th} and obese above the 95^{th} percentile. However, there is an argument that since these percentiles were arbitrarily chosen, they do not always relate to health consequences and may not represent individuals from all the countries. In addition, International Obesity Taskforce (IOTF), led to the development of international definitions for overweight and obesity in children based on reference data from six countries; UK, Brazil, Hong Kong, the Netherlands, Singapore and the USA (Cole et al. 2000). Through these definitions, quasi-centiles were produced that are linked to the adult overweight and obesity definitions (25 and 30kg/m2) (Cole et al. 2000). These cut off points for body mass index for overweight and obesity are age and sex specific and represent childhood obesity categories defined to pass through body mass index of 25 and 30 kg/m2 (Table 1.2).

However, there is criticism for both national and international reference data. For example, it is argued that for IOTF BMI definitions, although being used in paediatric research and epidemiology, the relationship of BMI and morbidity are very likely to be ethnic and population specific (Reilly 2002; Reilly 2005). In addition, Reilly et al. highlighted that IOTF definitions may underestimate the prevalence of obesity due to the fact that it was less sensitive than the national reference data when used in British populations (Reilly 2005). In a different study, international definitions presented large obesity differences between UK preadolescent boys and girls compared to the national reference data (Chinn and Rona 2002). There is also the argument that since the IOTF obesity definition was developed using reference data of developed countries, it does not represent developing countries. Considering the above criticism regarding the use of BMI as a measure of obesity, most epidemiological studies refer to the national reference data of percentiles to define childhood obesity since they provide meaningful health outcomes (Reilly et al. 2003). In general, the use of percentile cut-offs to define overweight and obesity in children can be challenging when studying prevalence and secular trends, as even

though they are age and sex specific, other parameters may influence the findings. For example, country BMI references collected in different years will represent prevalence of overweight and obesity with different percentile references. In addition, it can be argued that a more detailed study in the percentiles per se is necessary, in order to have more precise and representative findings regarding children's overweight and obesity prevalence.

Table 1-2 International cut off points for body mass index for overweight and obesity by sex between 2 and 18 years, obtained by averaging data from Brazil, Great Britain, Hong Kong, Netherlands, Singapore, and United States.

	Body mass index 25 kg/m²		Body mass index 30 kg/m²	
Age (years)	Males	Females	Males	Females
2	18.41	18.02	20.09	19.81
2.5	18.13	17.76	19.80	19.55
3	17.89	17.56	19.57	19.36
3.5	17.69	17.40	19.39	19.23
4	17.55	17.28	19.29	19.15
4.5	17.47	17.19	19.26	19.12
5	17.42	17.15	19.30	19.17
5.5	17.45	17.20	19.47	19.34
6	17.55	17.34	19.78	19.65
6.5	17.71	17.53	20.23	20.08
7	17.92	17.75	20.63	20.51
7.5	18.16	18.03	21.09	21.01
8	18.44	18.35	21.60	21.57
8.5	18.76	18.69	22.17	22.18
9	19.10	19.07	22.77	22.81
9.5	19.46	19.45	23.39	23.46
10	19.84	19.86	24.00	24.11
10.5	20.20	20.29	24.57	24.77
11	20.55	20.74	25.10	25.42
11.5	20.89	21.20	25.58	26.05
12	21.22	21.68	26.02	26.67
12.5	21.56	22.14	26.43	27.24
13	21.91	22.58	26.84	27.76
13.5	22.27	22.98	27.25	28.20
14	22.62	23.34	27.63	28.57
14.5	22.96	23.66	27.98	28.87
15	23.29	23.94	28.30	29.11
15.5	23.60	24.17	28.60	29.29
16	23.90	24.37	28.88	29.43
16.5	24.19	24.54	29.14	29.56
17	24.46	24.70	29.41	29.69
17.5	24.73	24.85	29.70	29.84
18	25	25	30	30

Source: Adapted from (Cole et al. 2000).

1.2.3 Other obesity measures in children

Apart from Body Mass Index there are other epidemiological methods for measuring obesity in children. Skinfold thickness is a measure of subcutaneous body fat that does not involve weight and is often used in epidemiological studies in children. This method usually involves the measurement of subcutaneous fat from four different sites of the body which are biceps, triceps, suprailiac and subscapular skinfolds. There are studies supporting that the sum of these four sites in children and more specifically triceps skinfolds in adolescents are better screening tool of obesity than BMI (Sardinha et al. 1999; Bedogni et al. 2003). However, there seems to be criticism in that these equations are often inaccurate and inclined to errors as they are population specific (Wells 2001). In addition, for a given BMI value, it has been shown that skinfold thickness varies by ethnicity, thus, it is important to consider differences in adiposity, when assessing disease risk in different populations (Sisson et al. 2009). There is also the argument of strong between-observer differences when measuring skinfolds and this may limit the accuracy of the collected data (Ulijaszek and Kerr 1999).

Another measure of obesity often used in epidemiological studies in children is waist circumference and it is considered a measure of central obesity. Moreover, it is well known that central obesity is an important risk factor of cardiovascular disease in adults (von Eyben et al. 2003). Scientific evidence shows that waist circumference is a better predictor of cardiovascular disease risk factors in children than BMI, however, further studies are necessary to determine more accurate cut off points for this predictive obesity measure. Interestingly, it is supported that the relative proportions of intra-abdominal and subcutaneous fat vary with ethnicity (Goran and Gower 1999), suggesting that waist measurement may have different health implications in certain ethnic populations. In conclusion, Body Mass Index remains the most widely used epidemiological measure of childhood obesity, despite the limitations that may have.

1.3 Obesity and Health Implications

Secular trends of obesity and diabetes have essentially occurred worldwide (Wang and Lobstein 2006). Obesity is associated with increased risk for several chronic diseases including diabetes, hypertension, heart disease and stroke (Field et al. 2001). Obesity is also associated with a range of diseases including gastro-esophageal reflux disease, colon cancer and liver diseases such as non-alcoholic fatty liver disease, cirrhosis and hepatocellular carcinoma (ACG 2008). Furthermore, obesity and overweight are associated with significantly reduced quality of life (Hlatky et al. 2010). Adams et al. reported that the risk of death during a maximum follow-up of 10 years is increased by approximately 20–40% in overweight individuals and 2.5-fold in obese individuals compared to normal weight individuals (Adams et al. 2006). However, it is essential to appreciate the health consequences of childhood obesity which are categorized into short- and long-term effects. Some of the health effects of childhood obesity include psychosocial ill health, asthma, hypertension, dyslipidemia and insulin resistance as well as persistence of obesity and cardiovascular risk profiles into adulthood (Dietz 1998; Reilly 2005).

There is conformity among experts in the field, who believe that it is mainly environmental factors such as overconsumption of energy-dense foods and reduced total energy expenditure rather than biological factors which fuel the obesity epidemic. Moreover, studies highlight the importance of maintaining high levels of physical activity from youth to adulthood in that it may have a significant impact in reducing obesity in adulthood in both sexes (Yang et al. 2007). Once more, this finding stresses the importance of the environmental factors which influence the predisposition to obesity from a young age, and later in life as adults. In addition, children classified as overweight, it is important to monitor weight status often, as studies have identified risk of later obesity. Longitudinal data by Reilly et al. at the University of Glasgow, showed a risk of later obesity eighteen to twenty times higher in overweight children, compared to those of healthy weight at age of seven (Reilly et al. 2010). Furthermore, maintaining a healthy weight as a child has been found to reduce the risk of developing long term cardiovascular related health problems in adulthood. In the Young Finn study, they studied children and adolescents between the ages of eight and thirteen, and investigated specifically the intima-media thickness (IMT) in the carotid artery. They concluded that obesity incidences in youth are significantly associated with increased carotid artery intima-media thickness and decreased elasticity in adulthood (Raitakari et al. 2005). In addition, a cross-sectional study of children and adolescents between the ages of five and eighteen, reported that severely obese children and adolescents have lower health-related quality of life than children and adolescents who are healthy, and similar to those diagnosed with cancer (Schwimmer et al. 2003).

The fundamental cause of obesity is the actual energy imbalance between the energy intake through nutrition and the energy expended through physical activity and our lifestyle in general. As previously mentioned, obesity and overweight is directly associated with raised body mass index (BMI), which is an important risk factor for several chronic diseases and health implications such as cardiovascular disease, the leading cause of death in 2008 (WHO 2011). In addition, increased BMI is also associated with musculoskeletal disorders, and especially osteoarthritis, a highly disabling degenerative disease of the joints and some forms of cancer such as breast, colon and endometrial (WHO 2011). Childhood obesity, according to World Health Organization, not only has future health implications during adulthood but causes children to experience breathing difficulties, increased risk of fractures, hypertension, early markers of cardiovascular disease, insulin resistance as well as psychological effects (WHO 2011).

On the contrary, Lloyd et al. in a recent systematic review suggested that the observed associations between childhood obesity and adult blood pressure, carotid intima–media thickness or cardiovascular events largely reflected the tracking of BMI from childhood into adult life, and concluded that there was little evidence of an association that was independent of adult BMI (Lloyd et al. 2009). The data suggested that not being overweight during childhood failed to provide any protection against the effects of obesity in adulthood, and that those who were obese as children but went on to become normal weight adults were not at any greater risk of cardiovascular disease (CVD). Interestingly, the most susceptible to the adult obesity risks and

particularly with respect to high blood pressure, were the lean children. In addition, a different study also observed that the strongest predictor of blood pressure was a change from being at the lower end of the BMI scale in children, to the higher end in adulthood (Lauer and Clarke 1989). A more recent study reported that the effect of adult BMI on blood pressure was highest in those who had been in the lowest BMI levels as children (Li et al. 2007).

Two studies have reported positive associations between childhood BMI and adult total cholesterol, (Lauer et al. 1988; Freedman et al. 2001) however, only one adjusted for adult BMI, and by doing so the direction of the relationship was reversed (Freedman et al. 2001). These findings suggest that those with higher BMI in childhood had lower total cholesterol in adulthood if they were no longer overweight. Similar themes emerged regarding associations between adult triglyceride concentrations and childhood BMI, with negative association observed in the two studies that made the adjustment for adult BMI. Although the above associations remain unexplained, they indicate the complexity in the relationship between obesity and metabolic risk across the lifespan, which is worthy of further study. BMI and lean body mass seem to be important contributors across the lifespan of metabolic risks and the cumulative effect of changes in body composition over time.

In addition, it appears that obese and overweight children are more likely to become obese adults, thus increasing disabilities and mortality chance from a premature age. Scientific evidence in a large Danish based-population study on 276,835 children has shown that higher childhood BMI values elevated the risk of having a coronary heart disease (CHD) event in adulthood. Each 1-unit increase in BMI z-score, at every age from 7 to 13 years in boys and from 10 to 13 years in girls, significantly increased the risk of an event. The associations became stronger with increasing age during this period of childhood. As children are becoming heavier worldwide, their findings suggest that more children are at risk of having a CHD event in adulthood (Baker et al. 2007). A similar increasing pattern was found in the study of Finnish men (Eriksson et al. 2001). As children become older, the BMI distribution widens, and the increase in weight required for a 1-unit increase in BMI z-score at 13 years of age is more than double of that at 7 years. It has been speculated that, aside from the fact that body size in late childhood is more proximal in time to adult body size, increases in BMI z scores at these later ages could reflect a greater accumulation of fat, in particular intra-abdominal fat, which increases the risk of CHD (Zimmet et al. 2007). Comparisons with other studies confirm these associations. Two early studies, one in the United States (Abraham et al. 1971) and the other in Sweden (Mossberg 1989; DiPietro et al. 1994), found indications that heavier children were at greater risk for cardiovascular disease. A British study of 2399 children aged 2 to 14 years reported an association between childhood BMI and death from ischemic heart disease (Gunnell et al. 1998).

However, higher BMI and excess of body fat are not the only obesity related health characteristics present during childhood. Investigations of the risk factors for CHD have shown that hypertension, dyslipidemia, impaired glucose tolerance, and vascular abnormalities are

already present in overweight children (Berenson et al. 1998; Tounian et al. 2001; Sinha et al. 2002; Weiss et al. 2004; Viner et al. 2005). In addition, higher body weight in childhood is associated with the presence of these risk factors in children (Weiss et al. 2004), and this association suggests a plausible mechanism relating higher childhood BMI with an increased risk of adult CHD. However, in the last couple of decades, a new cluster of risk factors have been identified and defined as syndrome X or metabolic syndrome. In 1988, Reaven and colleagues (Reaven 1988) described "the metabolic syndrome" as a link between insulin resistance and hypertension, dyslipidemia, type 2 diabetes, and other metabolic abnormalities associated with an increased risk of atherosclerotic cardiovascular disease in adults (Levitt and Lambert 2002). Studies suggest that the metabolic syndrome may originate in uterus (Levitt and Lambert 2002; Ozanne and Hales 2002). However, there is evidence that epigenetic programming may have a role in connecting environmental exposures with gene expression in relation to obesity and metabolic syndrome. 'Obesogens' are chemical compounds that can promote obesity by increasing the number of fat cells, by changing the amount of calories burned at rest, by altering energy balance to favor storage of calories and by altering the mechanisms through which the body regulates appetite and satiety (Janesick 2012). Moreover, obesogens are predicted to act prenatally by eliciting epigenetic modifications that alter the expression of key genes in adipogenic pathways (Janesick 2012). There are epigenetic changes that occur during germ cell development and can potentially lead to trans-generational effects that may persist for many generations after the initial exposure (Skinner 2010; Skinner et al. 2011). It is conceivable therefore, that epigenetic programming may be associated with adiposity and metabolic syndrome; however many more studies would be needed to strengthen the evidence and elucidate this relationship.

By definition metabolic syndrome is defined as a constellation of risk factors including obesity, dyslipidemia, impaired glucose metabolism and elevated blood pressure; all of which are major predictors for cardiovascular diseases (Grundy 2006; Grundy et al. 2006). In 2005, the International Diabetes Federation (IDF) published its definition of the metabolic syndrome in adults (as shown in Table 1.3), which describes the new International Diabetes Federation (IDF) criteria for defining metabolic syndrome in adults. However, in 2007 a new definition was proposed by Zimmet to assess risk or outcomes in children and adolescents.

Table 1-3 The new International Diabetes Federation (IDF) definition of metabolic syndrome in adults.

According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have: **Central obesity** (defined as waist circumference* with ethnicity specific values) **plus any two of the following four factors:**	
Raised triglycerides	≥ 150 mg/dL (1.7 mmol/L) **or specific treatment for this lipid abnormality**
Reduced HDL cholesterol	< 40 mg/dL (1.03 mmol/L) in males < 50 mg/dL (1.29 mmol/L) in females **or specific treatment for this lipid abnormality**
Raised blood pressure	systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg **or treatment of previously diagnosed hypertension**
Raised fasting plasma glucose	(FPG) ≥ 100 mg/dL (5.6 mmol/L), **or previously diagnosed type 2 diabetes** If above 5.6 mmol/L or 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome.

** If BMI is >30kg/m², central obesity can be assumed and waist circumference does not need to be measured.*

Source: Adapted from International Diabetes Federation, 2006.

The new children and adolescent definition is simple and easy to apply in clinical practice (Table 1.4). Waist measurement is the main component. Percentiles, rather than absolute values of waist circumference have been used to compensate for variation in child development and ethnic origin. The definition is divided according to age-groups: age 6 to 10, 10 to 16, and 16 or older. IDF suggests that the metabolic syndrome should not be diagnosed in children younger than 10, but that a strong message for weight reduction should be delivered for those with abdominal obesity. For children aged 10 or older, metabolic syndrome can be diagnosed with abdominal obesity (using waist circumference percentiles) and the presence of two or more other clinical features (elevated triglycerides, low HDL-cholesterol, high blood pressure, increased plasma glucose). Although some of these features in addition to body size and proportions change with age and development, in the absence of contemporary definitive data, the criteria adhere to the absolute values in IDF's adult definition. The exception is that one (rather than a sex-specific) cut-off is used for HDL. For children older than 16, the IDF adult criteria can be used.

Table 1-4 The new International Diabetes Federation (IDF) definition of metabolic syndrome in children and adolescents.

Age group (years)	Obesity* (WC)	Triglycerides	HDL-C	Blood pressure	Glucose (mmol/L) or known T2DM
6–<10	≥90th percentile	Metabolic syndrome cannot be diagnosed, but further measurements should be made if there is a family history of metabolic syndrome, T2DM, dyslipidemia, cardiovascular disease, hypertension and/or obesity.			
10–<16 Metabolic syndrome	≥90th percentile or adult cut-off if lower	≥1.7 mmol/L (≥150 mg/dL)	<1.03 mmol/L (<40 mg/dL)	Systolic ≥130/ diastolic ≥85 mm Hg	≥5.6 mmol/L (100 mg/dL) (If ≥5.6 mmol/L [or known T2DM] recommend an OGTT)
16+ Metabolic syndrome	Use existing IDF criteria for adults, ie: Central obesity (defined as waist circumference ≥ 94cm for Europid men and ≥ 80cm for Europid women, with ethnicity specific values for other groups*) plus any two of the following four factors: • raised triglycerides: ≥ 1.7mmol/L • reduced HDL-cholesterol: <1.03mmol/L (<40 mg/dL) in males and <1.29mmol/L (<50 mg/dL) in females, or specific treatment for these lipid abnormalities • raised blood pressure: systolic B‰ =130 or diastolic B‰ =85mm Hg, or treatment of previously diagnosed hypertension • impaired fasting glycemia (IFG): fasting plasma glucose (FPG) =5.6 mmol/L (≥100 mg/dL), or previously diagnosed type 2 diabetes				

WC: waist circumference; HDL-C: high-density lipoprotein cholesterol; T2DM: type 2 diabetes mellitus; OGTT: oral glucose tolerance test.
*The IDF Consensus group recognises that there are ethnic, gender and age differences but research is still needed on outcomes to establish risk.

Source: Adapted from (Zimmet et al. 2007).

Furthermore, childhood obesity is a major contributor to the elevated number of metabolic abnormalities and an increase in type 2 diabetes in adolescents (De Ferranti and Osganian 2007). Thus, the remarkable increase in prevalence of overweight and obesity in children all over the world has as a consequence the emerging type 2 diabetes not only in adults but in youth as well.

Obesity in children appears to relate not only to metabolic health but also to sociocultural and psychological issues. In recent years, the association between psychosocial consequences and overweight and obesity in children has been investigated, and there seems to be an interesting correlation between the two. For example, it has been found that overweight and obese children often face stigmatization and discrimination and as a result their psychological well-being is compromised (Wardle and Cooke 2005). In addition, depression appears to be another psychosocial consequence associated with overweight and obese children (Lobstein et al. 2004; Wardle and Cooke 2005) according to longitudinal studies. Childhood obesity has therefore multiple health implications that may also impact adulthood health and by understanding the

mechanism behind childhood obesity will aid not only in the treatment but more importantly in the prevention of this epidemic disease.

1.4 Obesity aetiology: a multifactorial problem

Obesity has been proven to be a multifactorial problem and is a health condition with multiple etiologies. Several factors contribute to the increase of the epidemic obesity effect, and thus require excellent planning of intervention and implementation strategies in order not only to treat, but also prevent it. Environment plays a key role in the development of childhood and adult obesity, with multiple subcategories, such as nutrition, socioeconomic status and physical activity. Overall, studies support that certain aspects of physical and environmental factors can shape behaviours of children in relation to obesity.

1.4.1 Physiological and health implications of nutritional factors: a potential risk factor for obesity pandemic

Nutrition is the intake or consumption of food in order to fulfill the dietary needs of our body. A good and well balanced diet provides one with adequate necessary nutrients, thus building a strong immune system and helping to develop and maintain physical and mental wellbeing. A non-balanced diet can have opposite effects and may lead to several types of deficiencies, malnutrition such as that of obesity and other related health conditions. In relation to childhood obesity however, it is important to distinguish the role of parents in the development of the children's dietary preferences, dietary patterns and nutritional behaviours.

Interestingly, it is not clear whether obesity develops due to an excess energy intake (EI) relative to energy expenditure (EE), a decreased EE relative to EI, or most likely, a combination of both. According to the literature, while young children appear quite capable of self-regulating their EI under unsupervised conditions (Birch et al. 1991; Shea et al. 1992), this regulatory capacity appears to be easily undermined by a variety of factors including the degree of parental control and attitude towards the child's intake (Lissau et al. 1993; Birch and Fisher 1998).

Studies support the idea/hypothesis that the dietary habits of children are affected by the parent's food related behaviours and preferences (Birch and Fisher 1998). In addition, parents act as role models and their feeding related practices are strongly associated with children's diet and weight gain (Faith et al. 2004). This highlights the strong responsibility of parents, and adults in general towards young children and their future health as adolescents and later as adults. Moreover, when young children move from childhood to adolescence they are considered more independent and autonomous, making the impact of the parenting practices and habits less powerful (De Bourdeaudhuij 1997). However in a cross-sectional study conducted on young adolescents, it was found that the parental intake and the household availability of fruits and vegetables as well as dairy products, was positively associated with adolescent consumption of these food groups (Hanson et al. 2005). This demonstrates that even though less dependent,

adolescents are still influenced by parent's healthy or not healthy behaviours and nutritional decisions. The collection of data for this study however, was conducted by telephone interviews with the parents of adolescents regarding the home food environment, eating habits and weight-related behaviours. Therefore, this may have caused over and underestimations of specific diet related behaviours of their children. Additionally, a recent longitudinal study found that not only does parental intake and restricting the availability of unhealthy foods affect the children's and adolescent's diets, but that also less permissive food related parenting behaviours may have a beneficial effect in adolescent's nutritional habits (Vereecken et al. 2010). Once again, the impact of parents on children/adolescent nutritional behaviour and additionally on the health impact of future adults seems to be an important factor, however further studies are needed in order to define the role of parents in children's health.

1.4.2 Physiological and health implications of a physical activity and inactivity: a potential risk factor for obesity

The description of physical activity is conventionally defined as ''any bodily movement produced by the contraction of skeletal muscle that increases energy expenditure above a basal level'' (HHS 2008). Physical activity is a complex construct that can be classified qualitatively into major categories of sedentary behaviours, locomotion, work, leisure activities, and exercise (Butte et al. 2011). The American College of Sport Medicine (ACSM), the American Heart Association (AHA) and the UK Chief Medical Officer, all recommend that adults participate in at least 150 minutes of moderate intensity physical activity (or at least 60 minutes of vigorous intensity physical activity) per week, to reduce the risk of cardiovascular diseases and T2D (CMO 2004; Sigal et al. 2006; Haskell et al. 2007). The most recent ACSM/AHA guideline recommended for adult individuals, is that they should undertake 30 minutes or more of moderate intensity physical activity during five days of the week or at least 20 minutes or more of vigorous intensity physical activity during three days of the week, as well as undertaking muscle strengthening exercises on two days of the week (Haskell et al. 2007). This updated the previous ACSM guideline by clarifying the definition of moderate intensity activities, incorporating vigorous physical activity and muscle strengthening activities, and specifying that a combination of these activities is complementary to the production of health benefits. Furthermore, children's physical activity involves different kinds of activities like playing games, chores, sports etc. and the World Health Organization provides different recommendations than the ones for adults. According to World Health Organization, children and youth aged 5–17 should accumulate at least 60 minutes of moderate- to vigorous-intensity physical activity daily in order to have beneficial effects on cardiorespiratory and muscular fitness, bone health, and cardiovascular and metabolic health biomarkers. In addition, it is recommended that for children most of the daily physical activity should be aerobic and vigorous-intensity activities should be incorporated at least three times a week (WHO 2012).

However, the participation rates in physical activity are not very optimistic in Europe. Data from European regions show that approximately 37% of people are insufficiently physically active and

particularly those from high-income countries (WHO/Europe 2010). Scientific evidence from surveys in 2005/2006 support that girls are less active that boys, across countries and age groups. Moreover, it was reported that 15-year-olds were less likely to report meeting the physical activity guidelines than 11-year-olds and interestingly, only 22% of the 11-year-olds, engage in moderate-to-vigorous physical activity for at least 60 minutes per day which is the recommended guideline (WHO 2008).

Levels of habitual physical activity and sedentary behaviour are now well established as important to both the current and future health of children and adolescents (Strong et al. 2005; Rey-Lopez et al. 2008) and attempts to reduce the decline in physical activity in adolescence have been the focus of many public health interventions in recent years (Stevens et al. 2007). Most studies of changes in physical activity during childhood and adolescence have been cross-sectional rather than longitudinal. In addition, most previous studies used subjective methods of measurement of physical activity, which do not provide accurate estimates of the amount and intensity of physical activity (Reilly et al. 2008). Longitudinal studies that have used objective methods (Baker et al. 2007; Jago et al. 2008; Nader et al. 2008) have usually measured change during adolescence rather than during childhood and have usually focused on girls (Baker et al. 2007; Davison et al. 2007; Pate et al. 2009) because of concern that declines in adolescent physical activity are especially marked in girls. Little is known about the timing, nature, and magnitude of changes in physical activity levels in childhood, and even less about longitudinal changes in levels of objectively measured sedentary behaviour. Moreover, results of older studies conducted before the epidemic of paediatric obesity may not provide evidence that is readily applicable to contemporary children and adolescents. Understanding the timing, nature, and extent of changes in physical activity and sedentary behaviour during childhood is crucial for the development of informed, effective, and evidence-based interventions aimed at preventing obesity and promoting cardio-metabolic health.

It is evident that the activity level of young children has decreased whereas sedentary time has increased (Reilly et al. 2004), especially through the adolescent years as children are more likely to be habituated to sedentary lifestyle (Riddoch and Boreham 1995). Furthermore, school based interventions and community programs are important in order to promote physical activity and healthy lifestyle in early childhood, thus preventing and treating obesity epidemic in Europe.

It is well accepted that the increased rates of overweight and obesity in children and adolescents over the past decades have promoted serious public health concerns. Evidence has shown that overweight children are more likely to become overweight adults (Whitaker et al. 1997) and thus being more prone to chronic diseases, than normal weight children. Genetic factors are thought to influence obesity by interacting and responding in certain ways with new environmental conditions (Stunkard 1991), however physical activity or inactivity seems to be a better predictor of the obesity effect. A longitudinal study assessing the cardiovascular risk in young Finns study published in 1319 young children aged 9 and 18 years old, which were followed for 21 years,

found that physical activity reduces body weight in youth but was not directly associated with adult abdominal obesity in either men or women. The model accounted for 19% of abdominal obesity in men and 13% in women (Yang et al. 2007). Participation in and maintaining physical activity from youth into adulthood plays an important role in reducing obesity in adulthood. However, the present study has some limitations that need to be taken into account when interpreting the results. First, BMI and physical activity in youth varied with age. BMI increased, as expected with age, whereas physical activity increased during childhood but decreased during adolescence. It is possible that the BMI was affected by the changes in physical activity during and after puberty. Second, measuring physical activity by means of a questionnaire may influence the results. Third, possible changes in physical activity patterns of individuals during the follow-up might have influenced the results as the lever or frequency of physical activity might have changed.

A different study reported that children between the ages of three and five, spend 80% of their time in light to sedentary activities as they got older (Reilly et al. 2004). Evidence of recent longitudinal Gateshead Millennium Study which measured changes in physical activity and sedentary behaviour in 405 English children over a period of 2 years, found that daily volume of physical activity declined by 83 counts per minute over 2 years; the percentage of daily time spent in MVPA was low (IQR: -1.4 to 0.9) at baseline and declined by 0.3% over the follow up. The percentage of daily time in sedentary behaviour was high at baseline and increased from 78.0% to 81.1% of the day. The decline in MVPA and increase in sedentary behaviour were significantly greater in girls and in those with higher BMI z scores at baseline. The low levels of childhood MVPA and high levels of sedentary behaviour reported in this study present a major public health concern. These findings, if replicated in other groups of preadolescents, suggest that efforts to prevent declines in physical activity should be directed at children not just at adolescents (Basterfield et al. 2010).

The regional environment and more specifically the social environment has an outstanding role when determining physical activity, adiposity and motor skills of young children (Burgi et al. 2010). An important part in children's participation in physical activity is the access to recreational facilities and schools, presence of sidewalks and controlled intersections as well as access to destinations and public transportation (Davison and Lawson 2006). In addition, researchers believe that safety might be another key factor contributing to children’s and adolescent’s physical activity (Alton et al. 2007). As previously mentioned, environmental factors contribute significantly in the development or prevention of chronic conditions such as obesity. Physical activity has been proven to lower the risk of stroke, hypertension and depression as it has a protective effect on the obesity risk as well as on heart disease (by 30%), diabetes risk (by 27%) and some forms of cancer (by 21-25%) (WHO 2010b). Clearly, in more recent years children spend less time playing outdoors due to several different reasons, some have been mentioned previously but also due to the technology evolution. Recent data from the Canadian Health Measures Survey (Colley et al. 2011) suggest that only 7% of children and youth aged 6-19 years

participate in at least 60 minutes of moderate- to vigorous-intensity physical activity per day, thus meeting the current physical activity guidelines from Canada (Tremblay et al. 2011), the U.S.(2008), the U.K (Bull FC 2010), Australia (Okely AD 2008) and the World Health Organization (WHO 2010a). It has therefore become more evident that children nowadays find it more interesting to stay in, watch television and play video games than to spend time outside and one can argue that this is also more convenient to the parents.

However, it is necessary to understand the consequences of this matter and realize that it is not very encouraging for young children's health. Studies show a relationship between sedentary behaviours like TV viewing and video games with higher BMI in young children of both sexes (Berkey et al. 2000). Prior to the 1980s, very little consideration was given to sedentary behaviours and their health. However, In 1985, the first study to investigate the relationship between a sedentary behaviour, television viewing, and weight status was published (Dietz and Gortmaker 1985). This study examined the relationship between television viewing and weight status in children and adolescents following a cross-sectional and longitudinal approach, and using data from the National Health Examination Survey. Results indicated that television viewing was positively associated with the prevalence of obesity, both cross-sectional and longitudinally. This led to a growing body of research examining the mechanisms by which television watching as sedentary behaviour impacted weight status and obesity prevalence. Furthermore, accumulating evidence shows that, independent of physical activity levels, sedentary behaviours are associated with increased risk of cardio-metabolic disease, mortality, and a variety of physiological and psychological problems (Treuth et al. 2007; Katzmarzyk et al. 2009; Owen et al. 2009).

In general, it is observed that physical environment is greatly involved in children's behaviour and is important to take into account all the parameters, people and communities when creating interventions to reduce or prevent obesity epidemic. Furthermore, it is important to understand what exactly is behind the increased inactivity of young children and try to implement ways to resolve this important health related problem.

1.4.3 Assessment of Physical Activity and Sedentary Behaviour by Objective and Subjective Measures of Physical Activity

Physical activity levels can be monitored using different methods in order to assess the health behavioural habits of the population and their association with morbidity and mortality rates. Accurate assessment is required to assess current and changing physical activity levels within the population, and to evaluate the effectiveness of interventions designed to increase activity levels. However, the measurement of physical activity in epidemiological studies and surveillance systems is difficult due to its complex nature. Measurements of physical activity, and energy expenditure associated with it, comprise time (duration), number of sessions (frequency) and intensity. One of the assessment methods widely used is self-reporting questionnaires, which provide a subjective measure of physical activity. Questionnaires vary in

their complexity, time frame and type of activity that want to be assessed. From the large number of physical activity questionnaires available today, only very few of them have been used with more frequency in different populations, this make it available for cross-cultural comparison of physical activity patterns. One of the most popular subjective instruments is the International Physical Activity Questionnaire (IPAQ). This questionnaire was developed in an attempt to standardize the assessment of the prevalence of physical activity in different countries and cultures around the world. However, despite globally acceptable measurement properties, the results from some validation studies worldwide indicated reasonable validity of the IPAQ instrument when used to determine physical activity patterns in populations (Hagstromer et al. 2006).

A second approach to measure physical activity is through the implementation of objective measurement tools. Objective assessment tools have the ability to capture components of physical activity when subjective measures often are not able to determine accurately, such as unstructured activities (house work duties, or lower intensity activities). The most common instrument nowadays are the movement monitors such as accelerometers and pedometers, however, other more sophisticated techniques such as doubly labeled water has been also used to determine total energy expenditure, but due to its cost this technique is not used for large epidemiological cohorts (Lee 2009). From all these techniques, accelerometers are the most widely used objective tool to measure physical activity. They provide objective and accurate information about frequency, duration, intensity and patterns of physical activities including children and older adults. However, information regarding specific types of physical activity (i.e. upper body movements) are not always captured with these devices (I-Min Lee *et al* 2009).

Figure 1.3 The Actigraph accelerometer device.

1.4.4 Physiological and health implications of socioeconomic status: a potential risk factor for obesity pandemic

Health inequalities are believed to be associated with socioeconomic status (SES) and seem to differ across counties and populations. Most theories that explain these inequalities, relate indicators of socioeconomic status, with the individual such as income, educational attainment, or occupation (Smith et al. 1990). Socioeconomic environment influences occupation, lifestyle, and nutrition of social classes, which in turn would influence the prevalence of obesity, diabetes and CVDs. In addition, childhood and adult obesity is related with increasing wealth and food availability in developing countries (Sobal and Stunkard 1989; Wang 2001). However, in developed counties, this is not the case, as it has been found that women of lower SES are more

likely to be overweight or obese (Sobal and Stunkard 1989), thus the barometer of SES on obesity is not consistent across different populations.

The association between childhood obesity and SES is not fully understood due to the complexity of this relationship and the fact that many parameters could be involved. For example, a study in the US showed a degree of overweight and obesity in low income adolescents, but not in younger children (Wang 2001) however special attention is needed when trying to interpret such findings, as multiple factors may be involved such as family and community environments. In a different study in London, the prevalence of overweight and obesity was high in school students, with significant socioeconomic and ethnic inequalities, however, the pattern over time was not clear (Wardle et al. 2006). A different study conducted in Wales, on 3 year olds, suggested an increase of overweight/obesity in children from most-deprived areas compared with those from least deprived areas (Brunt et al. 2008). Furthermore, a study on children aged 3-13 in Northern England found a weak positive association between deprivation (measured by the Index of Multiple Deprivation – IMD71) and obesity, but when obesity was examined on a small area level, high obesity prevalence were present in both deprived and affluent areas (Edwards et al. 2009). In the study of obesity prevalence in children age 5-10 over 3 decades, using NSHG and HSE data, Stamatakis explored two measures of SES; income and occupation of the head of the household. Trends in obesity prevalence were shown to increase much more markedly in children from families of lower SES using both indicators, although the statistical relationship between SES and childhood obesity was only of borderline significance (Stamatakis et al. 2005)

These studies illustrate that the nature of the relationship between socio-economic status and obesity could differ by population. However, more data on children are required to elucidate the real contribution of socio-economic status on the prevalence of obesity status in different populations.

1.5 Genetics and complex disease

The previous sections of this chapter have outlined a number of lifestyle factors that clearly contribute to an increase in the risk of obesity. However, environmental and lifestyle factors do not seem to explain all of the variance in obesity increase. It has been hypothesized that gene variants that might have contributed to human survival in times of food scarcity may predispose us to obesity in the present day (Eaton et al. 1988). In addition, twin, family and adoption studies, have demonstrated high heritability of obesity and increased individual's risk of obesity when having obese relatives (Stunkard et al. 1986; Stunkard et al. 1986; Price et al. 1993).

1.5.1 Progress in Genetics of Obesity

For more than fifteen years the two major epidemiological approaches for identifying genetic loci for common traits were the candidate gene approach and the genome-wide linkage studies. Although the candidate gene approach became more efficient over recent years in identifying

obesity related genes, genome-wide linkage studies have not yet been successful (Loos 2009). Genome-wide association studies (GWAS) have replaced the genome-wide linkage studies and have been able to shed more light on the genetic contribution to common obesity and other diseases. Genome-wide association studies (GWAS) are now beginning to diminish, as most of the findings were related with very small variance being explained. More recently, researchers are using NGS technology to rescreen candidate genes in depth and find more variants which could possibly contribute to BMI and obesity risk among populations. However, the Human Obesity Map has been the most efficient resource listing genes related to specific obesity associated traits and enables researchers to review published studies (Rankinen et al. 2006).

The candidate gene approach relies on the understanding of the biology and pathophysiology that underlies the susceptibility to different diseases like obesity and is hypothesis-driven. In obesity studies, genes that are thought to have a role in regulation of energy balance are tested for associations with obesity related phenotypic traits. In the very beginning of the candidate gene approach, experiments and laboratory work was very expensive and therefore only a very small number of variants per gene were studied in association with obesity traits. However during the modern era, experiment costs have been reduced and the availability of better and more accurate resources such as International HapMap and dbSNP, have provided better insight in the area of genetic variation. It is worth mentioning that the latest update of Human Obesity Gene Map reported 127 candidate genes for which there is at least one study available suggesting positive association with obesity related traits. These genes were chosen as candidates based on their role in metabolic pathways and on evidence from animal studies, monogenic forms of obesity and genetic association studies (Rankinen et al. 2006). However, findings for 12 genes alone were replicated in ten or more studies: *ADIPOQ* (adiponectin), *ADRB2* (adrenergic b2 receptor), *ADRB3* (adrenergic b3 receptor), *GNB3* [guanine-nucleotide-binding protein (G protein), b polypeptide 3], *HTR2C* [5-hydroxytryptamine (serotonin) receptor 2C], *NR3C1* (nuclear receptor subfamily 3, group C, member 1), *LEP* (leptin), *LEPR* (leptin receptor), *PPARG* (peroxisome proliferator-activated receptor g), and *UCP1*, *UCP2* and *UCP3* (uncoupling proteins 1, 2 and 3) (Rankinen et al. 2006). Interestingly, although there are a substantial number of studies which show an association of these genes with obesity, many other studies have shown no association with obesity, and therefore, the overall conclusion for most of these genes remains ambiguous. Unfortunately, no recent report comparable to the Human Obesity Gene Map report has been published. This report was not published after 2006, due to lack of financial support. In addition, the large amount of data which started to get published by 2006 was difficult to be handled solely by the authors without supporting staff (Rankinen et al. 2006). In addition to the Human Obesity Gene map, a recent study collected a total of 1,736 obesity associated loci and created an obesity database, including 1,515 protein-coding genes and 221 microRNAs (miRNAs) collected from four mammalian species: human, cattle, rat, and mouse (Kunej et al. 2012). This centralized database aims to facilitate the development of comparative systems biology approaches to address this important health issue of obesity.

The most evident limitation of the candidate gene approach, which was more frequent in the past, is the small sample size in most studies which does not provide sufficient power to find effects of the size typically associated with complex traits and disorders. However, in more recent years this approach is able to be used in larger population sizes and also is an important initiative for meta-analyzing the already available published data.

The second approach is the genome-wide linkage studies which are hypothesis-generating and aim to identify new genetic variants which might relate to a disease of interest. This approach relies on how the participants relate to each other and examines specific chromosomal regions and possible disease traits across generations. Despite significant power, a meta-analysis of European origin was not able to locate an obesity or BMI locus convincingly (Saunders et al. 2007).

The most recent epidemiological approach is the genome-wide association study. This approach is very similar to the linkage studies previously mentioned, however this approach interrogates the entire genome without taking into account prior hypotheses and assumptions. A genome-wide association (GWA) study, is based on linkage disequilibrium (LD), it can be applied in larger sample sizes and is able to narrow down unsuspected disease associated loci. Thus far, more than 300 replicated associations for more than 70 common diseases and traits have resulted using the genome-wide association and is considered now the most revolutionary method in genetic epidemiology (Loos 2009). Interestingly, two high-density genome-wide association studies confirmed FTO (fat-mass- and obesity-associated gene) as the first gene incontrovertibly associated with common obesity and related traits (Vimaleswaran and Loos 2010).

Although the progress in the genetics of obesity as well as other diseases has been rapid over the last decade, there are demands and challenges which researchers need to work on before genetic profiles and therapeutic interventions can be achieved. Additionally, it is vital to appreciate the necessity of a good balance between the accuracy of measurements in the field along with substantial sample sizes, thus creating a satisfactory baseline towards successful science. During the past years, the progress in investigating variants that lead to predisposition to obesity has been slow and with very little success. The scientific methods used to identify alleles associated with common diseases were the candidate gene and the genome-wide linkage approach, which were methods that to a large extent did not succeed. The above approaches have led to the identification of a large number of variants for obesity related phenotypes, however only a very small number has been confirmed (Perusse et al. 2005). In addition, Genome-wide association approach is the latest most efficient method used in epidemiology and has led to rapid progress in the understanding of genetic variation and common diseases. GWA based research has revealed that obesity susceptibility loci in adults are already associated with anthropometric traits in children and adolescents, whereas other variants may differ with age, the cumulative effect size is similar (Li and Loos 2008). These observations suggest that genetic predisposition is also a factor in determining the obesity risk; however, as obesity is a multi-

factorial disease, it becomes more complicated by gene-environment interactions, where specific populations with different genotypes respond in a different way in particular environments.

1.6 Genetics of Obesity

As previously mentioned, obesity, similar to other epidemic conditions has a complex multifactorial origin involving multiple environmental and genetic parameters. What is very controversial is the fact that although the human genetic material (DNA) has not changed over time, more and more genetic studies demonstrate significant genetic contribution to the prevalence of obesity. The increased obesity incidences in populations with a constant gene pool substantiate the rising role of environmental factors in obesity (Damcott et al. 2003). This indicates the need to not only study the genetic influence on obesity but also to investigate how the environment influences the obesity as a condition and how it affects the genetic predisposition to common disease. Surprisingly, before 2007 no genetic variation had been unequivocally associated with increased obesity risk and BMI in populations, despite big efforts and attempts to use genome wide linkage and candidate gene association studies (Saunders et al. 2007). Family and twin studies have shown that genetic factors contribute 40-70% to the inter-individual variation in common obesity (Maes et al. 1997). Candidate gene and genome wide linkage studies have identified over the past 15 years a large number of genes but only very few of those have been robustly confirmed in subsequent studies (Loos 2009). Scientific evidence shows that the growing prevalence of obesity around the world is not directly due to genes associated with weight gain, but due to their ability to increase fat gain susceptibility in individuals exposed to specific environments (Maffeis 2000). Researchers claim that the genetic role in obesity is very complex, involving the interaction of multiple genes (polygenic) with relatively small effects which may be additionally influenced by numerous environmental factors such as nutrients, physical activity and smoking (Froguel and Boutin 2001).

1.6.1 Recent progress in the identification of genetic variants associated with Obesity-related phenotypes

Obesity is a common, multifactorial condition for which susceptibility is determined by the joint actions of genetic and environmental factors. The dramatic increase in the prevalence of obesity over the past two decades is most likely due to changes in diet and physical activity (Hill et al. 2003b). However, it is well recognized that hereditary influences also contribute significantly to the susceptibility to obesity (Loos 2009c).

The genetic contribution to obesity has been established through family, twin and adoption studies (Maes, Neale, and Eaves 1997; Stunkard, Foch, and Hrubec 1986). Twin studies have shown that genetic factors explain 40–80% of the variance in body mass index (BMI) and in risk of obesity (Herskind et al. 1996; Loos and Bouchard 2008c), while lower heritabilities have been reported for family (20–50%) (Luke et al. 2001; Rice et al. 1999) and adoption (20–60%) studies

(Stunkard et al. 1986). As part of the recent GWAS discoveries, individual genome-wide association studies were combined through collaborative efforts in order to increase sample size and power, to identify more common variants associated to obesity. The GIANT (Genomic Investigation of Anthropometric Traits) consortium is an international collaborative initiative that brings together research groups focusing on anthropometric traits from across Europe and the USA. Data from seven genome-wide association scans for BMI (*n* = 16,876) were combined in their first meta-analysis (Loos et al. 2008). Despite a quadrupling in sample size compared with previous studies, only *FTO* and one new locus (188 kb downstream of *MC4R, "near- MC4R")*, out of ten loci that were taken forward for replication, were unequivocally confirmed. The near-*MC4R* locus was identified in another study in 2,684 Asian Indians, and confirmed in 11,955 individuals of Asian Indian and European ancestry (Chambers et al. 2008). The effect size was the same in both ethnic groups but the frequency of the risk allele in Asian Indians (36%) was greater than in white Europeans (27%), which might explain why this locus could be identified with a relatively small sample of Asian Indians in the discovery stage.

A third effort to identify obesity genetics variants, involved an increased sample size of 32,387 of European ancestry from 15 cohorts GIANT consortium (Willer et al. 2009a) (Figure 1.4). Of the 35 loci identified in the first stage of the genome-wide scan, eight loci were firmly replicated in an independent series of 59,082 individuals. These included the previously established FTO and near-MC4R loci and six new loci: near-NEGR1 (neuronal growth regulator 1), near-TMEM18 (transmembrane protein 18), in SH2B1 (SH2B adaptor protein 1), near-KCTD15 (potassium channel tetramerisation domain containing 15), near-GNPDA2 (glucosamine-6-phosphate deaminase 2), and in MTCH2 (mitochondrial carrier homologue 2).

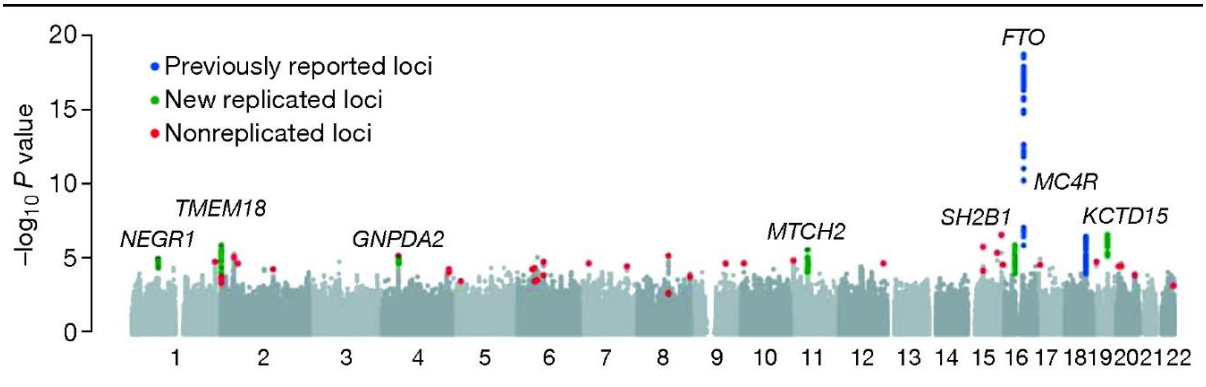

Figure 1.4 Manhattan plot showing the significance association of all SNPs in the stage 1 meta-analysis with BMI (GIANT consortium) (Vimaleswaran and Loos 2010).

Despite the discovery of all novel genetic variants associated to obesity, a recent study that genotyped the 12 obesity-susceptibility variants identified by the GIANT consortium and deCODE Genetics group in 20,431 individuals of a population-based study of white Europeans, showed that these genetic variants had a cumulative effect on BMI, with each additional risk-allele increasing BMI by 0.149 units, or weight by 444 g (Li et al. 2010a) (Figure 1.5).

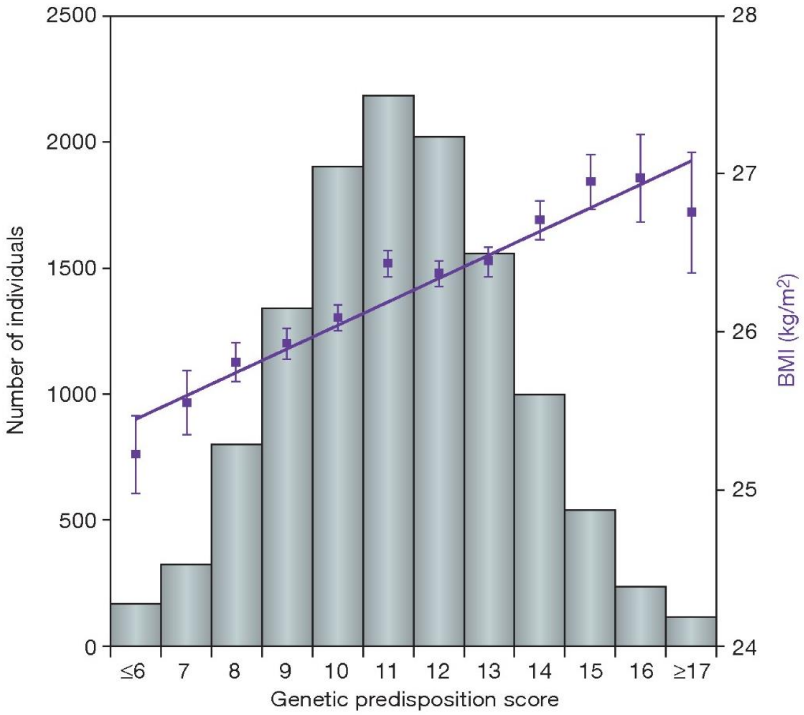

Figure 1.5 Cumulative effect on BMI of obesity-susceptibility variants.

The distribution of the genetic predisposition score and cumulative effects of the risk alleles of the 12 variants on BMI (mean ± SE) are shown (n = 12,201). Each additional allele is associated with an increase in 0.149 BMI units, or 444g in weight (Li et al. 2010b).

However, all these 12 obesity-susceptibility loci together explained less than 1% of the variation in BMI and had only limited predictive value of obesity (Figure 1.6). So far, the single effect of the genetic variant identified through GWAS for BMI is are associated with a small effect size on BMI (lying between 0.06 and 0.66 $kg.m^{-2}$ per each copy of the risk allele) and a risk of obesity ranging between 1.03 to 1.32 odds (Figure 1.5). These recently discovered genetic variants expose the substantial amount of missing hereditability in obesity that still remains unexplained (40–80%), if it is considered that all genetic variants have explained less than 1% of the BMI variation so far.

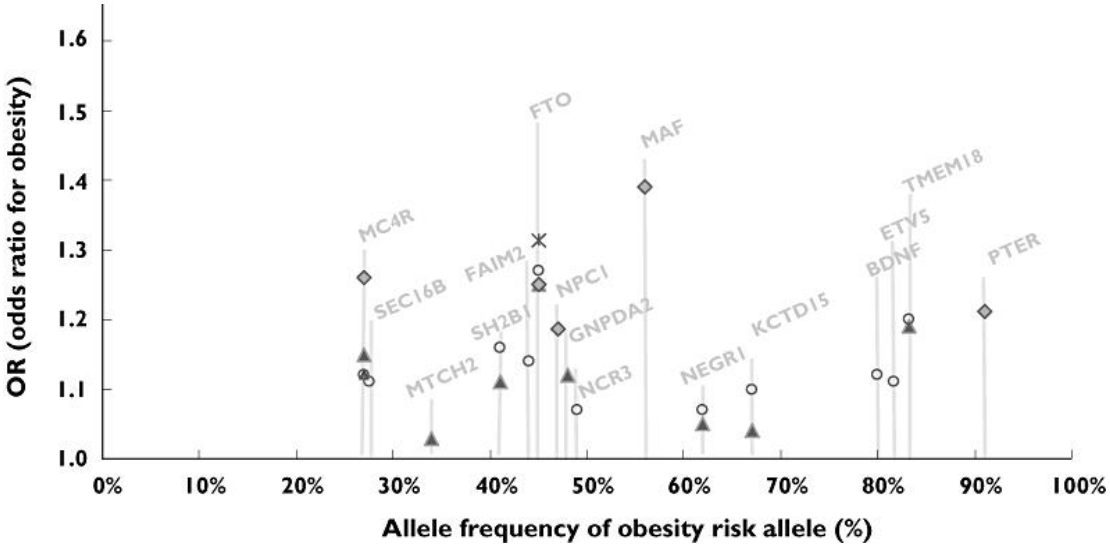

Figure 1.6 Effect sizes for risk of obesity reported for the established obesity-susceptibility loci (Loos 2009).

Effect sizes represent the increased odds of obesity for each additional risk-allele. ORs for PTER and NPC1 were inferred from the OR reported for the dominant model. (Frayling et al. 2007) (✳);(Loos and Bouchard 2008)(▲); (Willer et al. 2009)(▲); (Thorleifsson et al. 2009) (○); (Meyre et al. 2009)(◆).

1.6.2 *FTO* – Fat mass and obesity-associated gene

FTO is a large gene of nine exons spanning more than 400 kb on chromosome 16 in humans (Figure 1.7). The most commonly studied polymorphism (rs9939609) lies in the first intron of the gene (Groop 2007). All the SNPs identified so far are located in the first and largest intron of the gene, a region where the sequence is strongly conserved across species (Loos et al. 2008). This first intron contains 40 polymorphisms which are highly correlated in Caucasian populations (Loos and Bouchard 2008) as they are found to be in linkage disequilibrium (LD).

Although the gene's physiology and function is still elusive, studies involving bioinformatics analyses demonstrated that *FTO* is a member of the nonheme dioxygenase superfamily (Sanchez-Pulido and Andrade-Navarro 2007). *FTO* was also found to have a central role through an effect on cerebrocortical insulin sensitivity as subjects homozygous for the risk allele exhibit a reduced insulin response in the brain (Tschritter et al. 2007). *FTO* was also found to have a peripheral role in a study conducted in healthy women. The study demonstrated that *FTO* mRNA levels in adipose tissue increased with BMI and also that the lipolytic activity was reduced in the carriers of the risk allele independent of BMI (Wahlen et al. 2008). The functions of *FTO* mentioned above reveal the existence of a potential physiological mechanism by which *FTO* variants influence obesity.

Incidentally, *FTO* is present in all vertebrates and marine algae, but not in invertebrates, fungi, or green plants (Robbens et al. 2008). The *FTO* gene was actually identified in 1999, prior to the GWAS era, as one of six contiguous genes encompassed by a naturally occurring 1.6 megabase

deletion in a mouse model known as *fused toes* (*Ft*) (Peters et al. 2002). Using bioinformatics, Gerken et al., have proposed that the FTO protein is an Fe(II) and 2-oxoglutarate-dependent oxygenase (Gerken et al. 2007). The first complete *FTO* null mouse was reported in 2009; loss-of-function mutation in FTO caused and displayed a complex phenotype of postnatal growth retardation, decreased fat and lean body mass, increased metabolic rate, and elevated food intake when corrected for lean body mass (Fischer et al. 2009). In addition, a different mouse model with an ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis induced point mutation in *FTO* (I367F), resulted in a partial loss-of-function, it appeared with reduced fat mass and displayed increased energy expenditure (Church et al. 2009).

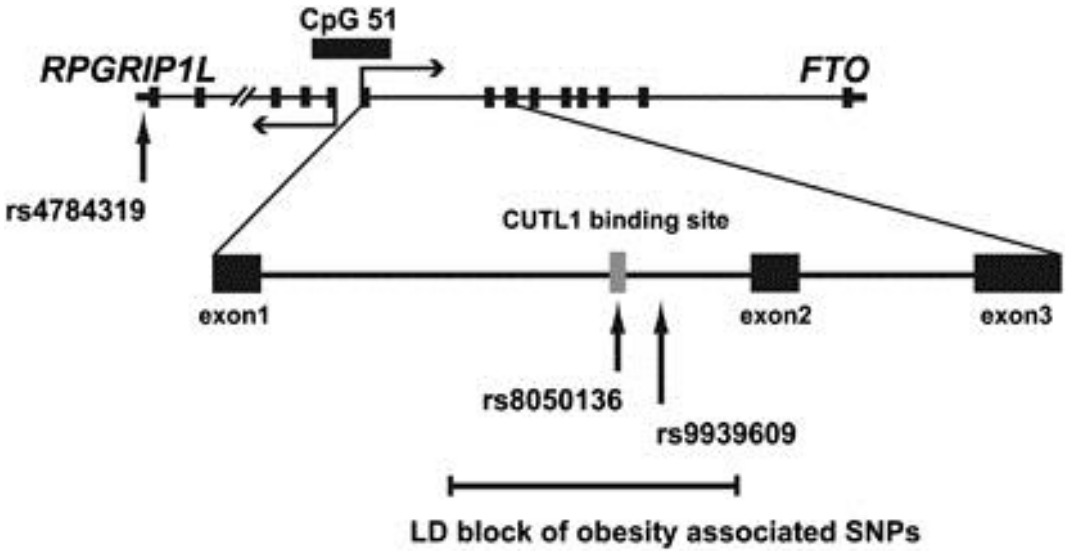

Figure 1.7 Physical map of the FTO and RPGRIP1L loci.

The FTO and RPGRIP1L genes are located on the long arm of chromosome 16, share a CpG island with 51 CpG dinucleotides and are transcribed in opposite directions. The obesity-associated SNPs are in strong LD and located within intron 1 of the FTO gene. The variants used in this study as well as the CUTL1-binding site identified by Stratigopoulos et al., are indicated (Stratigopoulos et al. 2008).

The *FTO* gene shares a CpG island with the adjacent RPGRIP1L gene, which is transcribed in the opposite direction, suggesting that the two genes are co-regulated. The RPGRIP1L protein is located in the cilia and centrosomes (Delous et al. 2007). Loss-of-function mutations in RPGRIP1L cause Joubert syndrome type 7 or Meckel syndrome type 5. It can be noted that FTO and RPGRIP1L genes are ubiquitously expressed and show similarity of expression profile both in fetal and adult tissues.

As the obesity-associated SNPs in the *FTO* gene are intronic, their functional significance is unclear. It is possible that one of the SNPs is located in a regulatory sequence and that the risk allele increases or decreases the transcription rate, but strong linkage disequilibrium (LD) of these SNPs makes it difficult to identify the functionally relevant SNP. In 2008, Stratigopoulos et al., reported that the risk allele (A) of SNP rs8050136 preferentially bound *to* the transcription

factor *CUTL1* in human fibroblast DNA and that an siRNA knockdown of CUTL1 by 70%, decreased *FTO* and *RPGRIP1L* expression by 90 and 65%, respectively (Stratigopoulos et al. 2008). However, these findings are not consistent with their model in which *FTO* and/or *RPGRIP1L* mediate suppressive effects on energy intake. To fit the normal model, *CUTL1* should preferentially bind to the non-risk allele of rs8050136. Furthermore, several studies have failed to reveal any influence of the *FTO* genotype on total mRNA level of *FTO* or *RPGRIP1L* (Kloting et al. 2008; Wahlen et al. 2008; Grunnet et al. 2009). Recently, an association between intronic variation of the *FTO* gene and transcript levels of the retinoblastoma-like 2 (RBL2) gene, which maps 270 kb upstream of *FTO*, was reported (Jowett et al. 2009). One problem in interpreting the previous findings is that the CCAAT-displacement activity of CUTL1 was implicated in the transcriptional repression of several genes, whereas some *CUTL1* isoforms were found to participate in the transcriptional activation (Sansregret and Nepveu 2008). Another problem is that studies on cis-regulatory effects on gene transcription in humans are hampered by the fact that the tested individuals unavoidably differ in genetic background, age, life events and environment. To detect subtle differences in transcript levels, very large numbers of individuals would have to be tested. These problems can be circumvented by determining the ratio of allelic transcript levels in heterozygous individuals, in whom each allele serves as an internal control for the other (Yan et al. 2002; Serre et al. 2008). For this approach, only few subjects are needed.

So far it has been reported that *FTO* is widely expressed across multiple tissues (Frayling et al. 2007; Gerken et al. 2007; Fredriksson et al. 2008) although it is most highly expressed in the brain and especially in the hypothalamus, an area that plays a key role in the control of energy homeostasis (Coll et al. 2008). Furthermore, in experiments with mice, *FTO* expression was shown to increase by ~60% in the hypothalamic area (the arcuate nucleus) in the fed state compared with mice in the fasting state (Gerken et al. 2007), suggesting a role of *FTO* in appetite and satiety regulation.

1.6.3 *FTO* and Obesity Risk

The discovery of the *FTO* gene and its potential role in obesity related conditions has provoked a series of studies aimed at better understanding obesity; a polygenic and multi-factorial health problem. It is well known that obesity is a serious international health problem and a major cause of morbidity and mortality, highly associated with metabolic syndrome, type 2 diabetes, heart disease and certain forms of cancer. Genetic studies have investigated *FTO* and its association with obesity and diabetes and although their findings and conclusions may vary, they are yet essential.

The first genome-wide association study of European descent, conducted by Frayling, showed that SNPs in the *FTO* gene region were strongly associated with Type II diabetes and the risk allele was also associated with increased BMI (Frayling et al. 2007). Furthermore, cross-sectional studies support that *FTO*'s effect on T2D is known to be mediated by its effect on BMI in

Europeans because the effect on T2D disappears when BMI is included in the model. *FTO* variants explain only 0.5-1.3% of the BMI variance (Frayling et al. 2007; Scuteri et al. 2007). In addition, a genome-wide association study demonstrated that approximately 16% of European descent individuals who were homozygotes for the risk allele weighed about 3kg more than controls (Dina et al. 2007; Frayling et al. 2007). This may suggest a role of *FTO* on appetite regulation and energy intake. Loss of *FTO* in mice was found to lead to postnatal growth retardation and significant reduction in adipose tissue thus protecting from obesity symptoms; however, according to the researchers this was due to increased energy expenditure rather than hyperphagia (Fischer et al. 2009). Another genome-wide association study compared extremely obese young individuals and healthy lean controls, and tested 15 SNPs out of which six were located in the *FTO* gene, showing genome-wide significance and association with obesity traits in nuclear families with at least one obese offspring (Hinney et al. 2007). Scutteri et al. after conducting a large genome-wide study, of BMI showed that variants in the *FTO* and PFKP gene were strongly associated with BMI and only those in the *FTO* were replicated in European and Hispanic Americans (Scuteri et al. 2007).

Risk haplotype of *FTO* was found to yield a proportion of population attributable risk of 22% for common obesity (Dina et al. 2007), supporting the idea that *FTO* contributes significantly to the prevalence of obesity and thus warrants further investigation. Furthermore, studies support that *FTO* gene was not only identified in genome-wide association studies in Caucasians but it also appears to act as a susceptibility factor in other populations as well. A recent study found *FTO*, among other genes, to be significantly associated with BMI and visceral fat area in a Japanese population (Hotta et al. 2010). Additionally, *FTO* was found to have an incidence of 24% increase obesity and T2D for each allele in a large European cohort (Cauchi et al. 2009), which highlights the need to accurately identify the prevalence of the risk allele in the general population thus determining their contribution towards public health. Interestingly, *FTO* was found to be associated with an atherogenic lipid profile and myocardial infarction in patients with T2D, and therefore, it may contribute in the future to more effective targeting of specific preventative therapy (Doney et al. 2009) However, despite the large amount of studies focused on understanding the physiological mechanism of the *FTO*, the actual function of the gene and how it influences obesity risk is still not clearly identified.

1.6.4 Gene and environment interaction and Obesity

Gene-environment interactions are a topic of tremendous importance since it aids in the understanding of the pathogenesis of multifactorial diseases such as obesity. In addition, the gene-environment relation can provide great improvement in the designing of appropriate intervention prevention strategies as well as design of personalized medication for target risk populations. Therefore, this area of research has a key role in the potential of prevention and the evolution of treating mechanisms and procedures of complex diseases.

Recent genetic association studies have identified gene-lifestyle interactions, as can also be seen for *FTO* gene. Up to date, gene-environment interaction studies in white populations have showed that the association between *FTO* variants and obesity-related phenotypes is more pronounced in individuals that are less physically active, whereas the association is diminished in those who have physically active lifestyles (Andreasen et al. 2008; Rampersaud et al. 2008; Vimaleswaran et al. 2009; Lee et al. 2010) . These observations suggest that genetic susceptibility towards obesity induced by variation in *FTO* can be overcome by adopting a physically active lifestyle; however this has not been shown in a prospective intervention trial. A recent meta-analysis on the influence of the interaction between the *FTO* rs9939609 SNP and physical activity on BMI in a random effects meta-analysis of 218,16 adults and 19,268 children was conducted (Figure 1.8). The magnitude of the effect of *FTO* risk allele on BMI was 30% smaller in physically active individuals (beta= 0.32kg/m^2) than inactive individuals (beta= 0.46kg/m^2). However, no such interaction was found in children (Kilpelainen et al. 2011).

Interestingly, a different study in Caucasian adults demonstrated that the *FTO* variant effect on BMI was not mediated by physical activity or energy intake, thus showing an effect on basal metabolic rate (BMR) per kg of body weight (Hubacek et al. 2010). This finding might be a plausible explanation for the *FTO* effect on body weight. In a different study, *FTO* was not found to be involved in the regulation of energy expenditure but it may affect the control of food intake and food choice, suggesting a link to a hyperphagic phenotype or a preference for energy-dense foods (Cecil et al. 2008). On the contrary, other studies conducted in children did not detect an association of *FTO* genotypes with dietary energy intake or physical activity (Berentzen et al. 2008; Scott et al. 2010). Moreover, the *FTO* protective allele was found to be protective against overeating by promoting elevated enjoyment to food and responsiveness to internal signals of satiety in children (Wardle et al. 2009).

In a study conducted in women in the U.S, lifestyle factors were found to modify the genetic risk of obesity phenotypes in those women with the risk *FTO* genotype, particularly in those who were inactive and had a higher caloric intake (Ahmad et al. 2011). In addition to the already mentioned interaction, there seems to be a different kind of gene- environment relationship among *FTO* and education. In a study of adult general Mediterranean population, an association was detected between *FTO* in non-university subjects whereas no association was observed in university subjects suggesting that education is able to modulate the association of *FTO* with BMI and obesity risk (Corella et al. 2010). This finding is in agreement with education being a proxy of healthier lifestyle, in that as people are more educated, they are accompanied by a cluster of healthier lifestyle factors (Deshmukh-Taskar et al. 2007; Nyholm et al. 2008; Rodriguez-Martin et al. 2009; Roskam et al. 2010). However, further research is needed before such conclusions are commonly accepted worldwide as similar lifestyle factors could influence health in opposite directions depending on the country's development status.

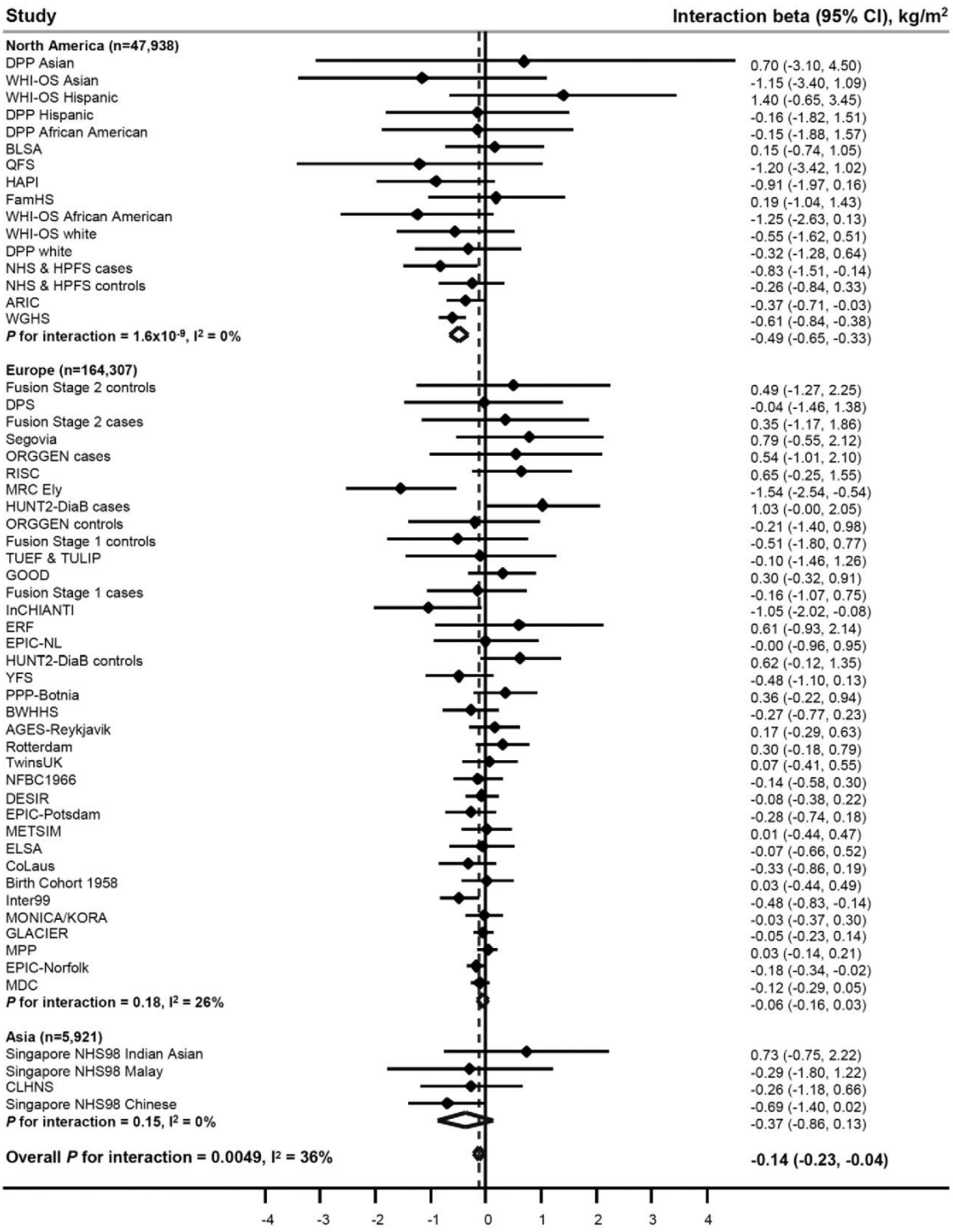

Figure 1.8 Forest plot of the effect of interaction between *FTO* rs9939609 SNP and physical activity on BMI in a random effects meta-analysis of 218,166 adults (Kilpelainen et al. 2011).

The studies showed in Figure 1.8 are sorted by sample size. The interaction beta represents the difference in BMI per minor allele of rs9939609, comparing physically active individuals to inactive individuals, adjusting for age and sex. For example, a beta interaction of 0.10 kg/m2 for BMI represents a 0.10 kg/m2 attenuation in the BMI-increasing effect of the rs9939609 minor allele in physically active individuals compared to inactive individuals.

Due to the multi-factorial origin of obesity and although *FTO* seems to put some children at greater risk of obesity, it is important to encourage a low dietary energy density intake which may be an efficient approach to help young children avoid excessive fat gain (Johnson et al.

2009). Understanding how genes and environmental factors interact and associate will help us better appreciate how lifestyle behaviours can modulate genetic contribution to risk of complex diseases such as obesity. Improving the knowledge regarding this area will greatly contribute to understanding the mechanisms behind the prevalence of obesity in specific populations.

1.7 Epigenetics in Obesity

Obesity appears to be a complex health condition with multifactorial etiology, including interactions of genetic background, environmental factors such as diet and sedentary lifestyle, and hormones (Martinez et al. 2008). The genetic revolution and the sophisticated genetic studies have enabled the identification of specific polymorphisms related to human obesity, and *FTO* has been the most replicated gene with the highest impact on obesity. However, despite these advances, the combined effect of all loci identified up to date, only account for approximately 2-3% of the inherited contribution to the obesity (40-70 %) (Marti and Ordovas 2011). Furthermore, researchers believe that the significantly remaining contribution of obesity's heritability, as well as the mechanisms by which environment could have an effect, may be due to epigenetic modifications (Marti and Ordovas 2011).

Epigenetics has been defined as the study of heritable changes in gene expression that occur in the absence of a change in the DNA sequence itself (Junien and Nathanielsz 2007). The epigenetic state of DNA and the related phenotype may be inherited in what is defined as trans-generational epigenetic inheritance (Morgan et al. 1999), where epigenetic modifications are not completely erased, and as a result, some of the epigenetic state is transferred to the next generation (Rakyan et al. 2003; Morgan and Whitelaw 2008). Interestingly, although the area of epigenetics is quite new, it has already been found to have an association with obesity and adipose tissue. There are three main epigenetic mechanisms that involve obesity related areas. The first to be identified is the Genomic DNA methylation, which is responsible for the gene expression in physiologic and pathologic states (Barres and Zierath 2011). DNA methylation seems to be associated in the regulation of specific genes involved in the glucose homeostasis regulation (Marti and Ordovas 2011). In addition, PPARG2, is an example of DNA methylation, which activates the adipocyte-specific genes expression, as it is progressively demethylated upon adipocyte differentiation (Fujiki et al. 2009). Potential genes that might be related to environmental and epigenetic modifications, that mediate the expression of key genes associated with adiposity are the *FTO*, MC4R and the PPARG (Herrera et al. 2011) The next epigenetic mechanism involves changes in chromatin organization by histone modifications. Studies have shown that histone demethylase (JHDM2a) has a protective role in obesity, since mice deficient in histone demethylase develop adult onset obesity (Inagaki et al. 2009; Tateishi et al. 2009). Lastly, epigenetic marks are the Noncoding RNSs or miRNAs, which normally bind to the target mRNA leading to translation inhibition and mRNA degradation (Marti and Ordovas 2011). The miRNAs are found to have a role in processes associated with adipocyte differentiation, insulin regulation and fat metabolism (Ortega et al. 2010; Hulsmans et al. 2011).

Epigenetic studies, although essential, are often constrained due to the high cost of the methylation chips, and thus the small size is causing underpowered results and findings. However, epigenetic mechanisms may aid in the understanding of inter-individual variation in disease risk such as obesity and there might be a stage which helps treat and prevent specific diseases. Moreover, it is essential for the careful design of studies to assess the epigenetic mechanisms and help establish the relationship between the genetic variation, epigenetics and complex diseases like obesity.

1.8 Summary

In summary, the literature review detailed in this chapter has explored the increasing prevalence of obesity in general and more specifically childhood obesity and the health implications as well as and potential factors that could explain the progression of this epidemic condition. The actual cause and etiology of obesity is complex and multifactorial and in most individuals is likely to be the consequence of the interplay between lifestyle and genetic factors. Evidence was described that supports the susceptibility to childhood obesity. Genetic factors could play an important role when young children are exposed to an obesogenic environment, which for example may promote sedentary lifestyle and unhealthy eating behaviours.

Thus, the hypothesis of this study is the following: European children may have a genetic background that makes them susceptible to the adverse consequences of obesity. Understanding the environmental influences such as physical activity patterns of this population, may help explain whether they can modulate or have a protective role over the genetically predisposing risk allele.

1.9 Aims

The overall aim of this study is to investigate the association between genetic and environmental factors such as physical activity on the predisposition to obesity in European children. The focus will particularly be on the description of this population and the obesity risk that is present during childhood, and on the characterization of the relative contributions of *FTO*, and environment and gene-environment interactions on the risk of obesity. FTO was chosen to be studied by the IDEFICS team as it has recently provided strong evidence of association in the general European population (Dina et al. 2007; Frayling et al. 2007; Scuteri et al. 2007). As most studies on FTO were conducted on adults, the author wanted to investigate the gene's association on European children (of 2 to 10 years old) and it's relation to other environmental factors such as PA. This investigation will also allow a comparison between sexes, age categories and countries. These findings will add more evidence to the understanding of this complex interaction between genes and lifestyle factors and their relationship with indicators of childhood health. To achieve this overall aim, the study will address the following aims:

To characterize anthropometric and physical activity profiles in European Children involved in the IDEFICS project (Chapter 3).

To determine whether the association between physical activity and adiposity-related traits differs between boys and girls in European Children involved in the IDEFICS project. (Chapter 4).

To investigate the association between the *FTO* gene and obesity-related phenotypes in European Children involved in the IDEFICS project (Chapter 5).

To investigate how physical activity levels modulate the association between genetic variation in *FTO* and obesity-related phenotypes in European Children involved in the IDEFICS project (Chapter 5).

2 General Methods

2.1 IDEFICS Study

The IDEFICS (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants) is the largest epidemiological study which targets on preventing childhood obesity and related disorders. IDEFICS is a European project funded by the sixth Framework Program of the European Commission and focuses on the critical ages of 2 to 10 years. The two major aims of the IDEFICS project were a) to enhance the knowledge of the health effects of an altered environment of children in Europe (i.e. changing the diet and environmental and lifestyle behaviours) and b) to develop, implement and validate specific intervention approaches in order to reduce the prevalence of diet- and lifestyle- related diseases in Europe. In this large European project, research Centers and Universities of eight European countries were involved including Germany, Italy, Spain, Cyprus, Sweden, Hungary, Belgium and Estonia with Germany being the coordinating country (Figure2.1).

The main vision of IDEFICS in line with the WHO European Charter on Counteracting Obesity is to promote a healthy environment for childhood and adolescent development, thus fostering a sustainable prevention of obesity and other nutrition and lifestyle related conditions.

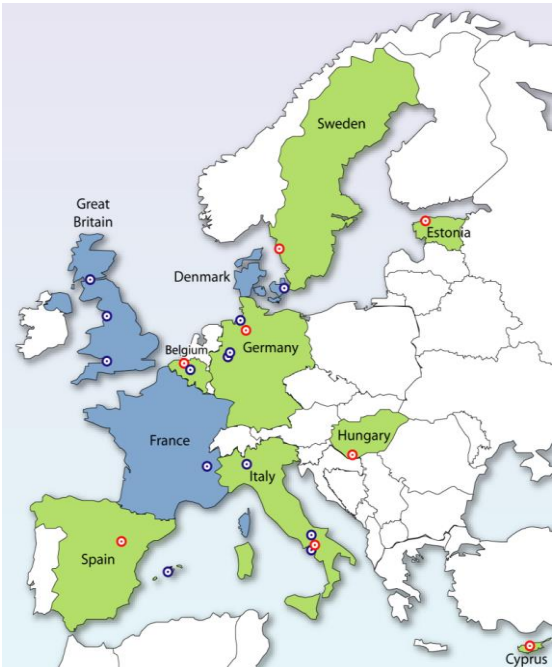

Figure 2.1 Geographic locations of all participating countries in the IDEFICS project.

In green all countries with survey centers where the data collection took place. In blue are countries that did *Complementary Research (*Denmark, France, and United Kingdom).

2.1.1 Study Design, Subjects and Population: IDEFICS

The IDEFICS (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants) uses a European multicenter approach to address health effects of obesity in young children. The IDEFICS study follows case-control approach in order to address etiological obesity health conditions and uses controlled intervention trials to attempt/investigate prevention of childhood obesity. The main focus of this dissertation is on the baseline survey cross-sectional module and not the intervention. In order to assess the feasibility of all instruments and procedures in field work and modify any measures that would give misleading results, extensive pretesting training was necessary (Suling et al. 2011).

A community-based approach was used to recruit subjects through schools and kindergartens, which facilitated all monitoring activities. A total of 16,224 children aged 2-10 was recruited from eight European countries (Sweden, Germany, Italy, Spain, Cyprus, Estonia, Hungary and Belgium). Prior to participation, the parents of the participating children were informed about the study though a letter and a consent form which they were asked to sign. According to the European Commission ethics consensus, each child participating in the IDEFICS study had to be verbally informed about the physical examination to follow using a simplified text.

All procedures and survey modules were completed following standardized protocols in all countries between September 2007 and May 2008. Apart from measurements and examinations, a parental questionnaire was used to assess information like gestational, behavioural, socio-demographic factors and the Children's Dietary Habits (CEHQ). The main examination categories were all standard anthropometrics measurements, genetic analyses (collection of genetic material from saliva samples), clinical parameters (blood and urine collection, blood pressure) and accelerometry to assess physical activity (Ahrens 2010). In small sub-samples other additional measurements were done such as physical fitness tests, taste perception, food preference and ultrasonometry of the calcaneus to asses bone stiffness.

The inclusion criteria, in order for the data to be considered valid during analysis were the completion of the parental questionnaire and the measurement of weight and height of the child. Initially, 31,543 children were invited to participate in the IDEFICS study. From the 16,864 individuals who submitted the consent form and participated, 16,224 met the inclusion requirements, and this leading in a participation rate of 51%.

2.2 Data Collection and Genetic Analysis

In this section the two major procedures will be described in detail. Firstly, the data collected in the field from all countries involved will be described, which includes anthropometrics, body composition, and physical activity measurements. Next, the biological material collection will be described as well as the genetic analysis which took place in University of Glasgow (UGLW) and in ISA-CNR, a molecular Laboratory in Italy.

2.2.1 Field Work Data Collection in all Centres

It is very important to note that the field work data collection was standardized and followed in the same way in all seven countries involved. The countries in which the data collection took place were Germany, Italy, Spain, Cyprus, Estonia, Hungary, Sweden and Belgium. Standard Operating Procedures were established for all physical examinations and procedures prior to data collection. The author of this thesis was practically involved during the data collection in Cyprus, where anthropometric, biological samples and PA data were collected. The author of this thesis was also responsible for the saliva collection and proper storage and safety of the genetic material. When the genetic material was transferred to the University of Glasgow, the author was responsible for the creation of IDEFICS bio-bank (approx.16,000 DNA samples), which involved proper sample management, purchase of storage equipment and detailed labeling of each sample that would enable accurate control of the study's genetic material. In addition, the author of this thesis performed the genotyping of eight SNPs of *ACE* and *ADRB2* (rs1042713 rs1042714 rs4351 rs4362 rs4329 rs4295 rs4353 rs4311) which were assigned according to the genetic panel of the study.

2.3 Anthropometry

Figure 2.2 Illustration of the field work data collection in IDEFICS participating centres.

2.3.1 Weight and Bioelectrical Impedance

Weight was measured using an adapted version of electronic scale (TANITA BC 420 SMA) to the nearest 50g. Children with cardiac pacemakers were strictly excluded from this measurement. The preferred status of the child before measurement of body fat was fasted, due to the fact that any food or drink consumption prior to the BIA measurement may influence accuracy and cause false estimations. Children where measured as lightly dressed as possible with bare feet and any kind of skin cream used had to be removed thoroughly prior to the measurement. Due to the fact that severe calluses or larger metallic implants might distort the BIA measurement in such cases, it had to be noted in the examination sheet.

2.3.2 Height

Height was measured using a stadiometer (SECA 225) and shoes had to be removed. Children who were not able to stand on the stadiometer were not measured. Any hair ornaments that might influence the measurement had to be removed and a second observer was preferably present to control correct positioning of the child during measurement. Children were measured standing on the stadiometer with feet slightly apart and back of the head, shoulder blades, buttocks, calves, heels touching the vertical board. The trained individual taking the measurement had to

ensure that the legs, the back and the head of the child were straight and that the feet were flat. The measuring slide was the pushed upwards according to the height of the child to be measured and measurement was recorded to the nearest 0.1 cm.

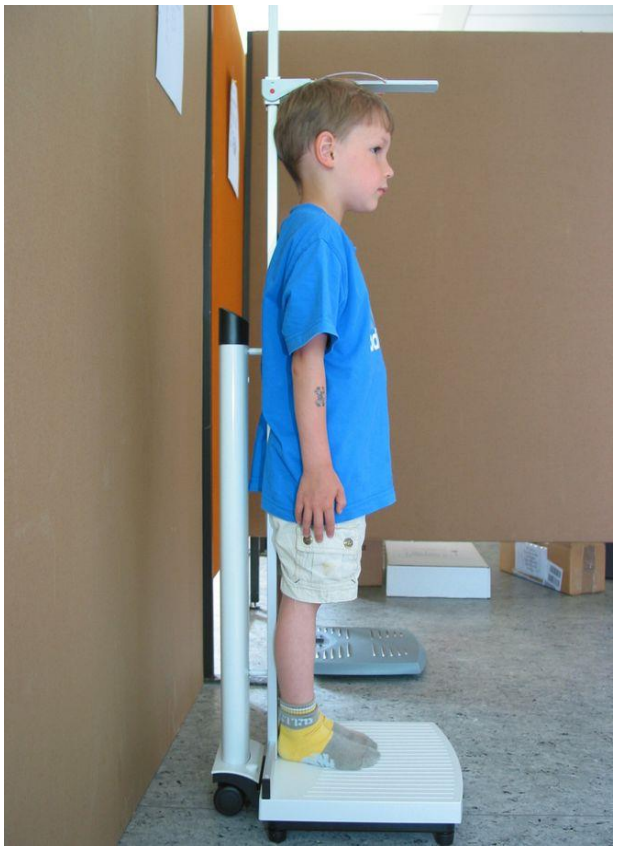

Figure 2.3 Illustration of the field work data collection of height in IDEFICS participating centres.

2.3.3 Body Mass Index (BMI)

BMI was calculated as the weight in kilograms divided by the square of the height in meters. Categorization was done using the Cole's criteria for categorizing overweight and obese children (Cole et al. 2000).

2.3.4 Skinfolds Thickness

All measurements of skinfold thickness were done using a skinfold caliper (Holtain Skinfold Calliper) and with children in a standing position. Measurements were done by trained individuals and at the right hand side of the body. Biceps and triceps skinfolds were measured at the midpoint of the right arm relaxed and the elbow flexed to 90 degrees and the palm facing superiorly. Measurement of subcutaneous tissue at the triceps and subscapular site were compulsory, whereas biceps and suprailiac were optional.

The length of the upper arm was determined by measuring the distance between the acromiale along the lateral side of the upper arm and the radiale. The midpoint was marked on the lateral side of the arm. Having the arm relaxed and the elbow extended and hanging just away from the side of the trunk and the palm facing the observer, the midpoint was marked after placing the tape perpendicular to the long axis of the arm. Subscapular skinfolds were measured with the upper extremities relaxed at the sides of the body. In order to locate the site, the trained individual palpated the scapula along the child's vertebral border until the inferior angle was identified. After the identification and marking of the inferior and lateral site of the scapula, the skinfold was picked 2cm downward the scapular marked point in a line 45◦ laterally downward. Suprailiac skinfold was recorded at the point 2cm above the iliac crest and 2cm towards the medial line. All the skinfold measurements were performed twice to the nearest 0.2 mm, and the mean of these two measures used.

Waist and Hip Circumferences were measured using a non-elastic tape-measure (SECA 200), with the child standing upright with the abdomen relaxed, the arms and sides. The tape had to be touching the skin but not compressing the soft tissues. Waist circumference was measured horizontally around the waist, at a point midway between the iliac crest and the lower coastal border, at the end of a gentle expiration. Hip circumference was measured at the point of maximal width around the hip region. The investigator recorded the measurements to the nearest 0.1 cm.

2.4 Physical Activity Assessment by Accelerometers

Physical activity patterns and sedentary behaviour were objectively measured using Actigraphs and Actitrainer (ActiGraph GT1M, ActiTrainer, Polar Heart Rate monitor strap). Prior to testing, each accelerometer was tested, fully charged and had the memory erased. Children with any physical handicap or constrained movement were excluded from this measurement. Each child had to wear a device during waking hours for three consecutive full days. Ideally, two of the days were weekdays and the other one weekend day. Parents were instructed to fill out an activity diary for the days the child would wear the accelerometer. This diary was used as a reference to monitor the children's compliance with wearing the device properly and for the time instructed; this data was not used in the analysis per se (Freedson et al. 2011). Parents also received an instruction document on how to use the device and were advised to remove the device from the children prior to bathing, swimming and showering as the device is not waterproof. The activity monitor had to be attached around the waist on the right hip side underneath the clothes immediately when the child got up and only removed when it was time to go to bed at night. The ActiTrainer was also worn around the waist on the right hip side and underneath the child's clothes; along with the heart rate monitor strap which was placed around the chest and below the chest muscles.

Physical activity patterns were calculated based on Sirard's physical activity cut-off points for children (Sirard 2005). Time spent in sedentary behavior was calculated using a cut-off point of <398 counts per 15 second, time spent in light intensity physical activities were estimated using a cut-off ranging between >398 and <890 counts per15 seconds), time spent in moderate intensity physical activity was estimated using cut-off point ranging between >890 and <1254 count per 15 seconds) and time spent in vigorous intensity physical activity was estimated using a cut-off point above 1254 counts per 15 seconds. Overall physical activity variable (average counts per minute, avg_cpm) was derived by adding the sum of the times spent in light, moderate and vigorous intensities physical activities during the three measurement days divided by the number of days (the average of this three days was used to calculate the cut-off point of all physical activity variables). Moderate to vigorous physical activity (MVPA) was derived by adding the sum of minutes spent in moderate and vigorous activities (this variable was expressed in minutes per day, as international guidelines on recommended level of physical activities for this specific intensity domain are expressed in minutes per day). Neither overall PA nor MVPA included time spent in sedentary behaviors. Valid accelerometer worn time was always accounted for time spent in different physical activity intensities in order to improve the accuracy of the activity or inactivity patterns. An international recommendation for physical activity measurement using accelerometers was used, and then at least 10 hours wearing time per day was setup as a valid measurement day (Freedson et al. 2011).

2.4.1 Accelerometer data analysis

Accelerometer data were analyzed using an automated method. After data collection, a summary sheet was generated by R software (version 2.9.0, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org). This summary sheet includes: total monitoring time, number of minutes per day spent on each of the physical activity intensities, which were estimated using the Sirard's cut-off point mentioned before. In order for non-wear time to be excluded from the data using the automated method, an algorithm developed using R was used which enabled R to automatically read in raw accelerometer files, re-integrate the data and to exclude invalid data. An exclusion criterion for determining non-wearing time was implemented, which involved the elimination of 20 minutes or more of consecutive zero counts prior to further analysis, as recommended by a standardized protocol in accelerometry measurement (Treuth et al. 2003) This report found that a period of 20 min or more of consecutive zero counts were not consistent with the awake state.

2.5 Assessment of Socio-economic Status, Educational Levels

Socioeconomic and demographic data of the parents were collected using a parental questionnaire. It was essential for the parents of participating children to complete the specific questionnaire during the period of their child being involved in the study in order for the collected data to be considered valid. Along with the SES data, the Level of education (LOE) was

also measured according to the International Standard Classification of Education (ISCED). Levels of education (LOE) were the following: Level 1 - Primary education or first stage of basic education, Level 2 - Lower secondary or second stage of basic education, Level 3 - (Upper) secondary education, Level 4 - Post-secondary non-tertiary education.

2.6 Collection of Genetic Material

2.6.1 Saliva Collection Methods and Storage

From the 16,223 included subjects, 14,019 (86.4%) provided a saliva sample during field work. The author of this thesis was involved in the sample collection in Cyprus (approximately 1000 samples). Approximately 2 ml of saliva was collected from children who were able to provide a sputum sample (Oragene DNA Self Collection Kit, tube format OG-300; DNA Genotek Inc., Canada), while sponges (Oragene DNA Self Collection Kit, disc format OG-250 and CS-1 sponge accessory; DNA Genotek Inc., Canada) were used to soak up as much saliva as possible from inside the mouths of younger children unable to spit. Measurements were undertaken by mobile field-testing teams that visited the schools and nurseries of the children, or when children visited a testing center. All samples were therefore collected under the supervision of trained personnel as subjects were typically too young to follow the instructions of the manufacturer. Prior to sample collection, children were advised to rinse their mouths with drinking water and to wait at least 5 min before providing a saliva sample. When using the saliva collection tubes, children were advised to spit into the tube until saliva had been collected up to the level indicated on the collection container (approximately 2 ml). Sponge samples were collected by a trained individual and were cut into the collection disk container according to the instructions of the manufacturer. Once collected, the trained individual was responsible for covering the tube or disk by placing the cap securely and inverting the container repeatedly for approximately 10 sec to allow the saliva sample to mix well with the Oragene chemistry (DNA Genotek Inc., Canada). Samples from each country were stored at room temperature (approximately 10 to 15 weeks) and subsequently couriered to the central laboratory at the University of Glasgow (UGLW) for DNA extraction, bio-banking and genotyping.

2.6.2 DNA processing/purification/extraction

Genomic DNA was extracted from a subgroup of 4,678 samples (Table 2.1). Samples collected using the sponge methods were available from 1,042 girls and 1,178 boys. Samples collected as whole saliva were available from 1,015 girls and 1,086 boys. Samples which were not recorded accurately regarding the saliva collection method were excluded from the total number of 4,678 (357 samples). According to the manufacturing company, DNA from saliva collected in Oragene containers should be stable for at least five years at ambient temperature and resists degradation even when stored at temperatures as high as 50°C (http://www.dnagenotek.com/pdf_files/PDPR012_LongTermStorage.pdf). Upon arrival at the

central laboratory at UGLW, samples were logged using a barcode reader system and stored at 4°C during processing.

DNA was extracted using the protocol for manual purification of DNA from saliva advocated by the manufacturer (http://www.dnagenotek.com/DNA_Genotek_Industry_AR_SCA_P.htmlwith) with minor adjustments to the protocol as detailed below. Prior to extraction, samples were incubated overnight at 50°C in an air incubator (Binder B28, BINDER GmbH, Tuttlingen, Germany). Following this, 500 µl of each sample was transferred into a 1.5 ml microcentrifuge tube and the remaining 1.5 ml sample was resealed in the original collection vessel and frozen at -20°C. Oragene DNA purifier (20 µl) was added to the microcentrifuge tube containing the sample, mixed by vortexing for a few seconds and then incubated on ice for 10 min. Following incubation, the sample mix was centrifuged using a microcentrifuge at room temperature for 10 min at 13000 rpm. The supernatant was carefully transferred with a pipette into a fresh microcentrifuge tube, 500 µl of 100% ethanol at room temperature was added and the tube mixed by inverting approximately 10 times. The tube was then allowed to stand at room temperature for 10 min to precipitate the DNA followed by centrifugation at room temperature for 2 min at 13000 rpm. The supernatant was then removed with special care and discarded with special care. When pellets were dried in an air incubator at 50°C for about 20 min they were taken up in 500 µl of TE buffer (100 mM Tris, 10 mM EDTA, pH 8.0). Samples were vortexed for a moment and stored at room temperature overnight to encourage DNA dissolution. All extracted samples were then stored at -20°C until quantification.

Table 2-1 Children fulfilling the inclusion criteria and providing saliva samples.

Country	n	% of total subjects[a]	Age Mean (SD)	Boys % (N)
Italy	1952	86.8	6.1 (1.8)	51.6 (1007)
Estonia	1418	82.5	6.0 (2.0)	49.3 (699)
Cyprus	1679	70.5	6.1 (1.4)	51.3 (862)
Belgium	1524	79.1	5.7 (1.6)	51.0 (777)
Sweden	1601	88.5	5.9 (2.0)	51.2 (820)
Germany	1947	94.2	6.2 (1.8)	51.2 (997)
Hungary	2518	98.1	6.3 (1.8)	50.0 (1260)
Spain	1380	91.6	5.9 (1.7)	51.1 (705)
All	14019	86.4	6.1 (1.8)	50.8 (7127)

2.6.3 DNA Quantification

Aliquots (167 µl) of each of the 4,678 successfully extracted DNA samples were transferred to 2 ml deep well plates (Starlabs UK Ltd, Buckinghamshire, UK) and quantified using the Nanodrop Technologies Nanodrop® ND-8000 Spectrophotometer (Wilmington, DE, USA) measuring 8 samples at a time, using a multichannel pipette to transfer 1.5 µl undiluted sample to the sample platform. DNA concentrations were estimated from absorbance readings at 260nm (A_{260}) using a 1 O.D. unit = 50 µg/ml conversion factor. In addition, A_{260}/A_{280} ratios were also measured. Values

for A_{260}/A_{280} ratio normally average approx. 1.8, with intact, high purity DNA usually having a ratio between 1.6 and 2.0. Ratios below approximately 1.6 indicate protein contamination and potentially reduced DNA stability and quality for polymerase chain reaction (PCR) amplification. Values above approx. 2.0 may indicate other small molecule/ionic contamination of the DNA solution. After a first round of quantification, DNA was diluted to a working concentration of 10 ng/µl in TE buffer in 2 ml 96 deep well plates. A re-reading was made to verify the target concentration (10 ng.µl-1) for each sample and subsequent aliquots were dispensed into 1 ml deep well plates (Starlabs UK Ltd, Buckinghamshire, UK) before storing the original samples in 2 ml plates at -20°C. The working samples were held at 4°C for several weeks during the genotyping analysis.

2.7 Genotyping Methods

2.7.1 TaqMan Genotyping: Overview

All the specific genes variants were genotyped with the ABI 7900-HT Sequence Detection System® (Applied Biosystems, Warrington, UK) using the TaqMan SNP genotyping assay for allelic discrimination. The assay contains the two primers for amplifying the polymorphic sequence of interest and two TaqMan MGB probes for distinguishing between the two alleles. Briefly, the assay takes advantage of the 5' to 3' nuclease activity of AmpliTaq Gold® DNA polymerase. This system uses a forward and reverse primer, and a probe that specifically targets each single nucleotide polymorphism (SNP) allele. The probe is labeled at its 5' end with two fluorescent dyes, VIC® dye is linked to the 5' end of the allele 1 probe whilst FAM™ dye is linked to the 5' end of the allele 2 probe, and at the 3' end with a non-fluorescent quencher. As long as these two are close to each other (i.e.: when they are on the intact probe), the quencher absorbs the fluorescence. During the Polymerase Chain Reaction (PCR), if the probe matches the sequence, the AmpliTaq Gold® polymerase moves along the region to be amplified in a 5' to 3' direction and cleaves the probe, separating the dye and the quencher. The fluorescence is no longer absorbed by the quencher and the fluorescence emissions generated by the PCR amplification are processed by the ABI 7900-HT Sequence Detection System® (SDS) (Applied Biosystems, Warrington, UK) that outputs genotypes with the allelic discrimination end-point analysis mode of the SDS software package, version 2.0 (Applied Biosystems, Warrington, UK).

The choice of genes to be genotyped was decided by the IDEFICS genetic expert panel. A thorough review of the literature was prepared regarding obesity related genes and a careful selection of polymorphisms was chosen, in which most were in Linkage disequilibrium with other worth noted SNPS. At University of Glasgow laboratory polymorphisms (SNPs) of ADRB2 and ACE genes (rs1042713 rs1042714 rs4351 rs4362 rs4329 rs4295 rs4353 rs4311) were assigned to be genotyped. For the purposes of this thesis, *FTO* was selected for testing its association with obesity-related traits and its interplay with environmental factors, such as physical activity in European children. Genotyping was performed in all 4,678 samples for eight single nucleotide polymorphisms (SNPs) from the β2-adrenergic receptor (*ADRB2*) gene (two assays) and from the

angiotensin I converting enzyme 1 (*ACE*) gene (6 assays) using Taqman® assays (Applied Biosystems, Warrington, UK). There were no DNA template controls included on each plate. Genotype calls were made by the analysis software (StepOne™ v2.1; Applied Biosystems, Warrington, UK). In order to analyze the genotyping success rate in relation to DNA quality, cutoffs were applied for A_{260}/A_{280} ratios at <1.5 and >2.1, and for DNA yields (expressed as natural log) at <0.5 or >5.0 (Figure 2.3). These cutoff points were arbitrarily chosen for being unusual in DNA of good quality produced by the methods applied, and for creating an 'outlier' dataset representing about 10% of the samples.

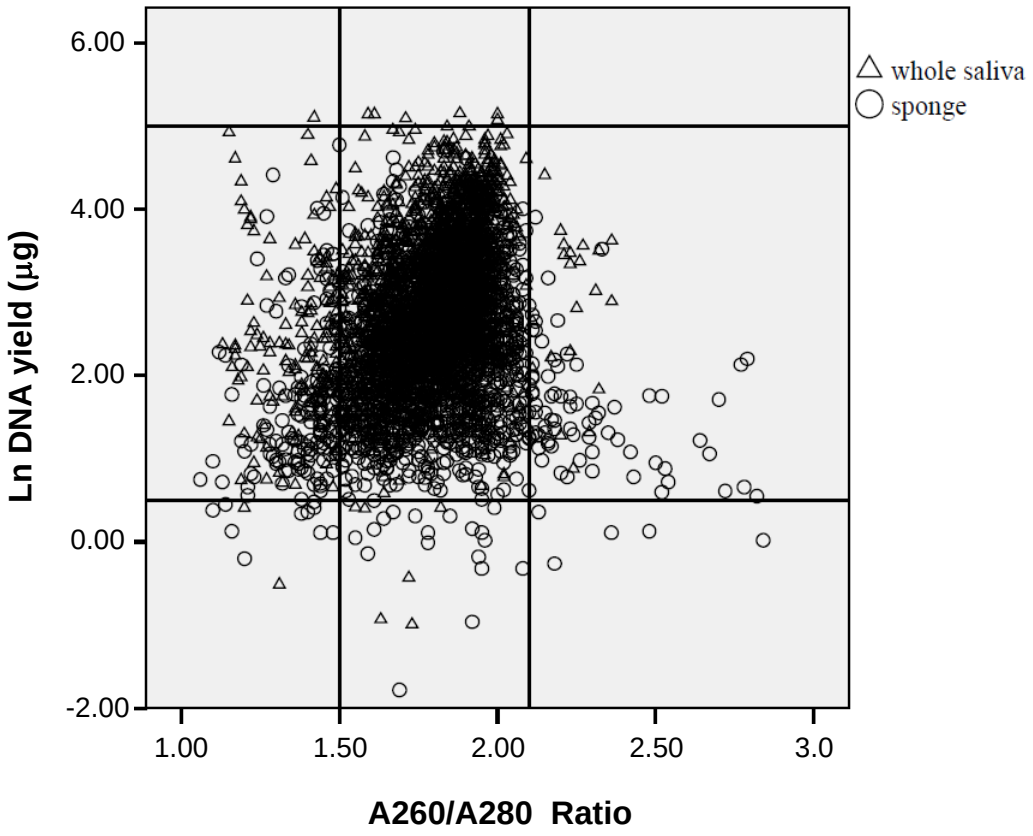

Figure 2.4 The relationship between DNA yield and quality from whole saliva and sponge samples.

A_{260} measurements were used to calculate DNA yield (µg) and then natural log transformed for presentation and plotted against A_{260}/A_{280} ratio by collection method. To separate outliers from the main cluster, four reference lines were applied. Vertical lines indicate A_{260}/A_{280} ratios of 1.5 and 2.1. Horizontal DNA yields of 0.5 and 5.

2.8 Polymerase Chain Reaction (PCR) Conditions and Endpoint readings

To genotype the specific gene variants a gDNA with a standardized concentration was used as per the manufacturer's instructions (Applied Biosystems, California. USA). To perform the Real-Time Polymerase Chain Reaction (PCR), a total final volume of 20 µl per well (with 2.0 µl of gDNA, 1.0 µl of TaqMan genotyping assay, 10.0µl of TaqMan universal PCR master mix and 7.0 µl of DNase-free water) as recommended for the ABI SNP genotyping protocol.

Each sample was dispensed into a definite labeled well in a specific PCR 96-well plate (Applied Biosystems, Warrington, UK). Each plate included 94 DNA reactions and two negative controls, where gDNA was replaced by DNase-free water and each sample was only found once on a single plate. The genotyping was carried out in the ABI 7900HT Fast Real-Time PCR device using the manufacturer's recommended PCR thermal cycling, which started with the enzyme activation (10 min at 95°C), followed by a denaturing step of 40 cycles at 92°C for 15 seconds. It was then completed by a 1 min extension step at 60°C.

After PCR amplification was performed an endpoint plate read using the Sequence Detection System (SDS) Software, which use the fluorescence measurements made during the plate read to plot the fluorescence (Rn) values based on the signal from each well. The plotted fluorescence signal indicates which alleles are in each sample. The SDS software was set up with the automatic allele calls system. All the information generated from the analysis was saved for posterior analysis in a excel file. The genotyping success rate for the eight SNPs is shown in Table 2-2. The unsuccessful genotypes were random over subjects and showed no tendency to cluster in particular individuals.

Table 2-2 Genotyping success rates in saliva samples.

Gene	FTO	ADRB2		ACE					
SNP	rs9939609	rs1042713	rs1042714	rs4351	rs4362	rs4329	rs4295	rs4353	rs4311
Successful genotype calls (%)	96	96	96	96	97	97	96	96	97

2.9 Statistical Analysis of the DNA quality

For the above genotyping results analyses were carried out using SPSS software package, version 15.0 (SPSS Inc., Chicago, USA) and MINITAB 15.1.30 (Minitab Ltd., Coventry, UK). Distributions of DNA yield and A_{260}/A_{280} ratio (by extraction method) were examined and normality assessed using the Anderson-Darling normality test. Non-normal distributions were transformed using the standard function (e.g. raw, log, inverse etc.) closest to optimal after Box-Cox analysis. Group differences for normally distributed (i.e. transformed) variables were tested using an independent t-test, with Welch's correction where variances were unequal (evaluated using Levene's test). For presentation purposes, mean values and other relevant statistics were back-transformed. Differences were considered statistically significant at $P<0.05$. Data are presented as mean ± SD/ or as median and interquartile range.

Specific statistical analyses used are described in detail within the method section of each chapter.

3 Description of IDEFICS population and obesity related phenotypes

3.1 Introduction

It is well recognized that childhood obesity has rapidly risen to become a serious chronic health problem and has reached epidemic levels in most developed counties. The frequency of overweight children (defined as BMI greater than 85th percentile for age and sex) has tripled over the last three decades (Thibault and Rolland-Cachera 2003). In 2008, more than one third of children and adolescents were overweight or obese (Ogden et al. 2010). Overweight and obesity in childhood appear to have tremendous physical (hyperlipidemia, hypertension abnormal glucose tolerance and infertility), (Dehghan et al. 2005) and psychological effects such as depression which occurs more frequently in obese children (Daniels et al. 2005). Therefore, although the real mechanism of obesity is not yet fully understood, it is undeniable that is a serious health condition with multiple causes and consequences. Early evidence shows that increased adiposity in childhood is predictive of obesity in adulthood (Whitaker et al. 1997; Parsons et al. 1999).

Childhood obesity appears to be an unsolved health problem in Europe and there is inconsistency in the obesity prevalence between countries and populations. The large variety of definitions and cut-off criteria of overweight and obesity, make -inter and -intra country comparisons of the obesity prevalence almost impossible (Livingstone 2001). In addition to this, the sample size of most studies is quite small and the randomness of the population chosen is often uncertain (Livingstone 2000) which leads to results with less power and misleading conclusions as the sample size and selection may not be representative of the population studied. Although longitudinal studies in children are essential to track the development of adiposity in children, most studies in the field are cross-sectional and as a result, a cause and effect relationship cannot be established, and this is also due to geographical variation.

It is important to identify the real etiologies responsible for this epidemic disorder, and environment and lifestyle preference and cultural environment appears to play major roles in the rising prevalence of obesity (Grundy 1998; Hill and Peters 1998). It is well acknowledged that lifestyle changes over the past decades are tremendously different than what was apparent in the past, thus resulting in a decrease in physical activity and increase in caloric intake (Miller et al. 2004). Studies demonstrate an important role of TV viewing in relation to prevalence of childhood obesity, as it has been linked with a rate of obesity that is 8.3 times greater in children who watch over five hours of television per day, compared with those who watched two hours or less per day (Proctor et al. 2003). It has been found that the national regulations on

programs directed to children could have an impact in the prevalence of obesity in European countries (Caroli et al. 2004). In addition, studies support that children spend more time watching television and playing on the computer than exercising (Saelens et al. 2002). Numerous studies have shown that the above sedentary behaviours are strongly associated with increased prevalence of childhood obesity (Swinburn and Egger 2002; Tremblay and Willms 2003). However, parents report they prefer their children to be in the house watching television, than playing outside unattended, thus being able to do other housework without worrying they are in danger (Gordon-Larsen et al. 2004). As a result, the amount of time children spend playing outside has diminished over the past few decades and physical education programs in schools have been reduced significantly (Livingstone et al. 2003).

Physical inactivity therefore is another strong contributor to childhood obesity and community and scientific interventions are essential to promote physical activity behaviours, in order to prevent childhood obesity from increasing over the years. The IDEFICS study offers a large number of samples and includes the critical ages of 2 to 10 years of age in European children. In addition, physical activity measurements were achieved with the use of acceleromenty devices, thus allowing for a more accurate collection of physical activity data.

The aims of the present chapter were: To characterize the anthropometric and physical activity patterns in European Children.

3.2 Research Design and Methods

3.2.1 Participants

A cohort of 16223 children aged 2–9 years old were recruited into a population-based baseline survey from eight European countries ranging from north to south and from east to west: Belgium (11.8%), Cyprus (14.6%), Estonia (10.5%), Germany (12.7%), Hungary (15.8%), Italy (13.8%), Spain (9.6%), and Sweden (11.1%). This recruitment was balanced by age (4y: 16.9%, 5y:15.4%, 6y: 14.1%; 7y: 16.2%, 8y; 22.2% and <10y:15.2%), sex (50.9% boys and 49.1% girls) and country. Sampling recruitment was related to the geographical location of the participating centers and all schools and kindergartens of each area where asked to be included and participate in the IDEFICS study. After meeting the inclusion criteria for genotyping (weight and height data available), a certain number of subjects were chosen for genotyping from each country. This sample selection was random and aimed for similar numbers of boys and girls from each country. The total sample selected for the genotyping studies was 4407 children, and was extracted from the whole IDEFICS population participating in the baseline survey. This sub-sample of children were genotyped for the assessment of the possible effect of *FTO* rs9939609 on each individual's BMI variation. The IDEFICS cohort provided at least 90% power to detect a 20% difference between AA and TT homozygotes at a significance level of a = 0.05, assuming an additive mode of inheritance. PA data was measured in half of the participants who were genotyped, as gene

and environment (physical activity) interaction was not the main aim of the project and the sample size with physical activity data was limited (Table 3-1).

During this research, all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed. Study subjects and their parents could consent to single components of the study while abstaining from others. Approval by the appropriate Ethics Committees was obtained by each of the 8 centers doing the field work and all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

3.3 Anthropometric Measurement

All anthropometric measurements were conducted in the participating schools of the study. Weight, Height, waist and hip circumferences and skinfolds at four sites (biceps, triceps, subscapular, and suprailiac) were measured following standard protocols mentioned in detail in sections 2.2.

3.3.1 Physical Activity

Physical activity patterns and sedentary behaviour were objectively measured using Actigraphs and Actitrainer (ActiGraph GT1M, ActiTrairner, Polar Heart Rate monitor strap). Prior to testing, each accelerometer was tested, fully charged and had the memory erased. Children with any physical handicap or constrained movement were excluded from this measurement. Each child had to wear a device during waking hours for three consecutive full days. Ideally, two of the days were weekdays and the other one weekend day. Parents were instructed to fill out an activity diary for the days the child would wear the accelerometer. Parents also received an instruction document on how to use the device and were advised to remove the device from the children prior to bathing, swimming and showering as the device is not waterproof. The activity monitor (Actigraph) had to be attached around the waist on the right hip side underneath the clothes immediately when the child would get up until and only removed when it was time they would go to bed at night. The ActiTrainer was also worn around the waist on the right hip side and underneath the child's clothes; along with the heart rate monitor strap which was placed around the chest and below the chest muscles. Physical activity patterns were calculated based on Sirard's cut off points.

3.4 Socio-economic Status, Educational Levels

Data on the socioeconomic status of the children was collected through a parental questionnaire. The variable describes the education status of the parent completing the questionnaire and can be found in appendix. . Along with the SES data, the Level of education (LOE) was also measured according to the International Standard Classification of Education (ISCED). Levels of education (LOE) were the following: Level 1 - Primary education or first stage of basic education, Level 2 -

Lower secondary or second stage of basic education, Level 3 - (Upper) secondary education, Level 4 - Post-secondary non-tertiary education.

3.4.1 Statistical analysis

Statistical analysis was developed using STATA (version 11; Statacorp, TX, USA) and Statistica (version 8.0, StataSoft, Tulsa, USA). Continuous data for all subjects were scrutinized for outliers using descriptive statistics and normal probability plots. Those individuals with any missing phenotype data were identified and excluded from the dataset. The trimming of the data was done using age and sex specific z-scores and by eliminating values above and below 3 SD from the mean. This procedure was done for all the quantitative variables which follow in this section.

Age categories are described in table 3.1. Means were compared using unpaired t-test for a sex-stratified analysis. Differences between more than 2 groups were tested using ANOVA and ANCOVA tests under a General Linear Model framework with posthoc pairwise tests to determine where differences lay. Posthoc tests were subject to Bonferroni correction for multiple testing where appropriate. Trend analyses were performed using a multiple regression analysis and coding age groups as ordinal numbers (0 to 8). The adjustments of continuous variables were applied using GLM or multiple regression models that include this type of variables. For those discrete or categorical variables, such as country, dummy code was used for coding them. This coding was applied using a specific command in STATA called "i." which allowed for the proper coding of dummy variables and included them in the models as categorical variables rather than continuous or ordinal. The same approach was used for all categorical or discrete variables in the thesis. For all analyses significance was accepted at $p < 0.05$.

3.5 Results

Descriptive summaries and a t-test were prepared to test whether the genotyped group differed from non-genotyped group in the main outcome variables (Table 3-1 and Table 3-2). The t-test between the two groups demonstrated non-significant p-values suggesting that the genotyped subset is representative of the full IDEFICS cohort rather than being a biased subset, with respect to these variables. A subset of 4407 participants was selected from eight European countries out of the total IDEFICS cohort. Table 3.1 shows a similar proportion between countries, ranging between 11% and 13.1% in both sexes. Participants for this study were aged between 2 and 10 years old. A similar proportion of participants were for all specific age groups (11 to 17%), except the age group of eight which included more participants of both sexes compared to the other age groups (23% to 24%).

Table 3-1 Sample size for each phenotype of the IDEFICS full cohort and the sub-cohort with genotype data.

	Full Cohort (n=16223) (Boys / Girls)	Genotyping Cohort (n=4407) (Boys / Girls)
Sex	8259 / 7964	2239 / 2114
Age	8259 / 7964	2293 / 2114
Birth weight (gr)	7746 / 7480	2242 / 2062
Height (m)	8221 / 7924	2282 / 2107
Body Mass (kg)	8139 / 7857	2268 / 2095
BMI ($kg.m^{-2}$)	8117 / 7851	2267 / 2097
Underweight [n (%)]	927 (11.2) / 838 (10.5)	258 (11.3) / 206 (9.7)
Normal Weight [n (%)]	5829 (70.6) / 5441 (68.3)	1650 (71.9) / 1485 (70.3)
Overweight [n (%)]	939 (11.4) / 1121 (14.1)	248 (10.8) / 293 (13.9)
Obese [n (%)]	564 (6.8) / 564 (7.1)	137 (5.9) / 130 (6.2)
Waist (cm)	7883 / 7604	2260 / 2094
Hip (cm)	7854 / 7619	2272 / 2101
Subscapular (mm)	7565 / 7322	2188 / 2025
Triceps (mm)	7631 / 7400	2206 / 2043
Sedentary time ($min.day^{-1}$) *	3330 / 3218	1063 / 949
MVPA ($min.day^{-1}$)*	3330 / 3218	1063 / 949
Vigorous activity ($min.day^{-1}$)*	3330 / 3218	1063 / 949
Overall Physical Activity ($count.min^{-1}$)*	3330 / 3218	1063 / 949

Table 3-2 Differences between the full cohort and the cohort with genotype data on continuous variables.

	Genotyping Cohort (n=4407) Mean ± SD	Full Cohort (n=16223) Mean ± SD	*P* value
Sex (Boys / Girls)	8259 / 7964	2239 / 2114	--
Age	6.1 ± 1.7	6.0 ± 1.7	0.090
Birth weight (gr)	3342.1 ± 569.9	3330.6 ± 568.1	0.243
Height (m)	1.17 ± 12.8	1.17 ± 12.3	0.869
Body Mass (kg)	22.8 ± 6.6	22.7 ± 6.7	0.307
BMI ($kg.m^{-2}$)	16.2 ± 2.1	16.3 ± 2.2	0.516
Underweight (%)	1765 (10.8)	464 (10.5)	0.956
Normal Weight (%)	11 270 (69.4)	3 135 (71.1)	0.812
Overweight (%)	2 060 (12.6)	541 (12.2)	0.873
Obese (%)	1 128 (6.9)	267 (6.1)	0.659
Waist (cm)	54.1 ± 6.3	54.2 ± 6.4	0.313
Hip (cm)	62.7 ± 7.7	62.7 ± 7.9	0.598
Subscapular (mm)	6.7 ± 2.9	6.8 ± 3.1	0.149
Triceps (mm)	11.0 ± 3.7	11.0 ± 3.8	0.588
Sedentary time ($min.day^{-1}$) *	614.0 ± 81.7	613.9 ± 87.8	0.979
MVPA ($min.day^{-1}$)*	10.6 ± 8.5	10.4 ± 8.4	0.217
Vigorous activity ($min.day^{-1}$)*	2.4 ± 2.8	2.4 ± 3.1	0.858
Overall Physical Activity ($count.min^{-1}$)*	602.2 ± 165.1	598.0 ± 164.4	0.316

Values are reported as mean and SD, except for those reported as prevalence, for which the number of cases and % are given. Chi-Square test was performed in order to estimate difference between frequencies in each BMI category group and t-test was performed for continuous variables.

Socioeconomic status was determined using level of parental education; from the total sample recruited just 54% had SES data. From those participants with SES data, Level of education (LOE) 1 and 3 had the lower proportions, while the highest number of the participants had a Level of education LOE 2 and 4 (Table 3.1). The descriptions of the age groups are also shown in table 3.1.

A summary of anthropometric and obesity related phenotype data is shown in table 3.2 There was no significant differences in age, body mass and BMI between sexes in unadjusted analysis, but girls were shorter in height and showed lower waist circumference with significantly higher hip circumference, subscapular and triceps skinfold than boys. This difference persisted after adjustment for age and country. However, following adjustments, the differences between sexes in body mass became significant ($p<0.0002$). Table 3.3 shows the cohort distribution by

nutritional status using Cole's classification. The major proportion of boys (72%) and girls (70.3%) where in normal weight status. However girls showed a slightly higher percentage of overweight and obesity than boys. No significant differences were found in distribution between boys and girls.

Table 3-3 Descriptive of demographic variables stratified by sex.

	Boys (n / %)		Girls (n / %)	
Sex (n=4407)	2293		2114	
Country				
Italy	290	12.6	276	13.1
Estonia	272	11.9	295	14.0
Cyprus	255	11.1	238	11.3
Belgium	291	12.7	248	11.7
Sweden	277	12.1	270	12.8
Germany	321	14.0	266	12.6
Hungary	291	12.7	258	12.2
Spain	296	12.9	263	12.4
Age categories				
<4 (2 to 4 years old)	404	17.6	351	16.6
5 (4 to 5 years old)	346	15.1	312	14.8
6 (5 to 6 years old)	260	11.3	247	11.7
7 (6 to 7 years old)	385	16.8	363	17.2
8 (7 to 8 years old)	534	23.3	507	24.0
<10 (8to10years old)	364	15.9	334	15.8
SES				
LOE - 1	174	7.6	168	7.9
LOE - 2	553	24.1	520	24.6
LOE - 3	185	8.1	170	8.0
LOE - 4	335	14.6	293	13.9

LOE: Level of education: Level 1 - Primary education or first stage of basic education, Level 2 - Lower secondary or second stage of basic education, Level 3 - (Upper) secondary education, Level 4 - Post-secondary non-tertiary education.

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Table 3-4 Anthropometric and obesity-related phenotypes characteristics in boys and girls.

	Boys		Girls		Unadjusted	Age-adjusted	Age & country- adjusted
	n	Mean ± SD	n	Mean ± SD	*p*-value	*p*-value	*p*-value
Age	2293	6.0 ± 1.8	2114	6.0 ± 1.8	0.434	-	0.452
Age squared	2293	39.7 ± 21.1	2114	40.1 ± 20.9	0.504	-	0.562
Height (m)	2293	117.9 ± 12.9	2114	117.0 ± 12.7	0.012	<0.0001	<0.0001
Body Mass (kg)	2268	23.0 ± 6.5	2095	22.7 ± 6.7	0.101	0.0002	<0.0001
BMI ($kg.m^{-2}$)	2267	16.3 ± 2.1	2097	16.2 ± 2.2	0.933	0.589	0.673
Waist (cm)	2260	54.3 ± 6.2	2094	53.8 ± 6.4	0.027	0.001	0.0003
Hip (cm)	2272	62.5 ± 7.7	2101	63. 0 ± 7.8	0.023	0.014	0.027
Subscapular (mm)	2188	6.3 ± 2.5	2025	7.3 ± 3.6	0.000	<0.0001	<0.0001
Triceps (mm)	2206	10.2 ± 3.4	2043	11.9 ± 3.9	0.000	<0.0001	<0.0001

Data presented as unadjusted mean ± SD. P-values are given for unadjusted, age-adjusted and age + country adjusted analysis for all variables, except age that was adjusted just for country.

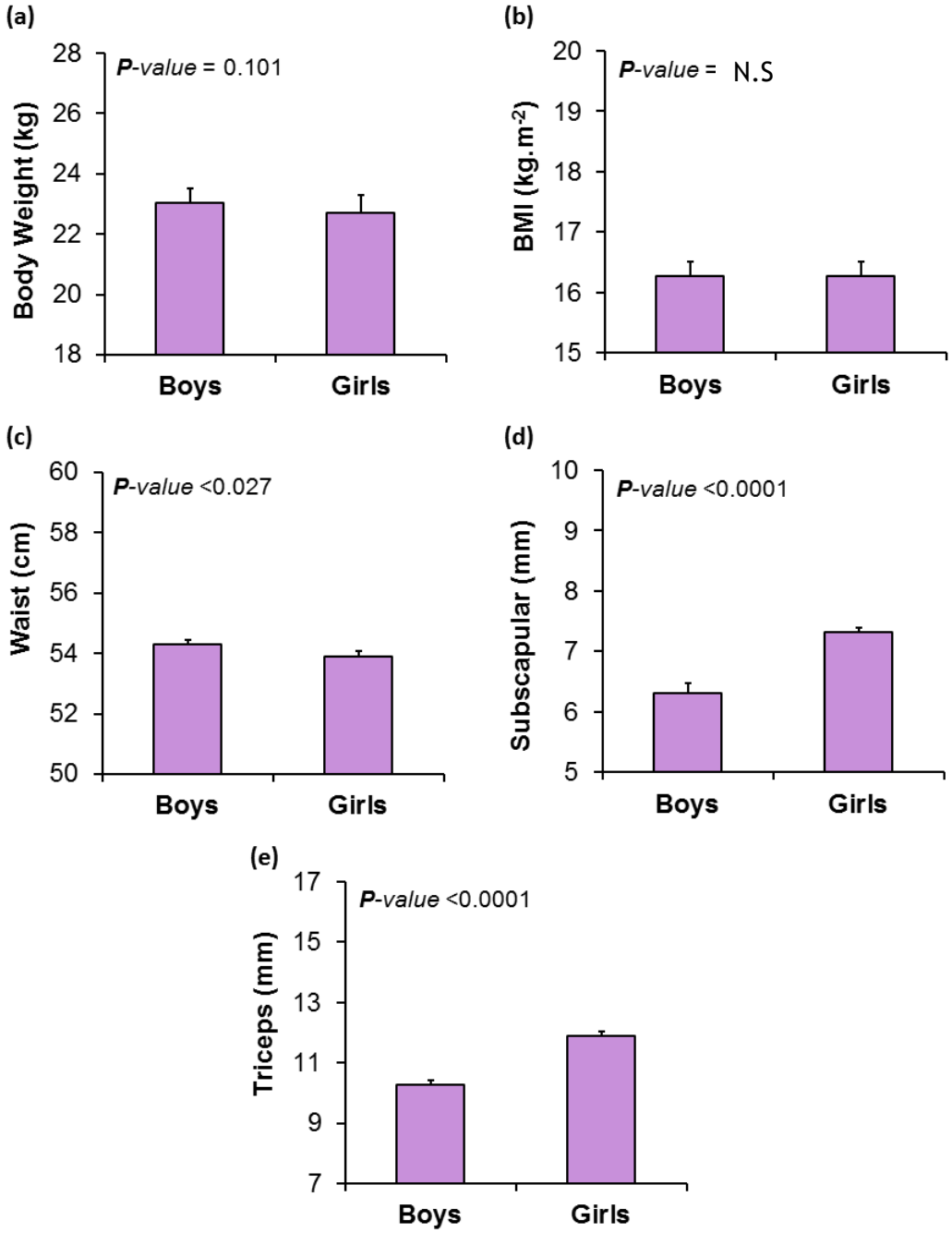


Figure 3.1 Obesity-related phenotypes characteristic in boys and girls.

Data presented as unadjusted mean ± SEM. P-values for differences between boys and girls were calculated for unadjusted data.

Table 3-5 Description of BMI categories in IDEFICS cohort.

BMI categories by Cole	Boys n (%)	Girls n (%)	P- value
thinness grade III	8 (0.3%)	6 (0.3%)	0.997
thinness grade II	37 (1.6%)	34 (1.6%)	0.981
thinness grade I	213 (9.3%)	166 (7.8%)	0.095
Normal Weight	1650 (72.0%)	1485 (70.3%)	0.072
Overweight	248 (10.9%)	293 (13.8%)	0.061
Obese	137 (5.9%)	130 (6.2%)	0.181

X^2 test was used to analysis differences in percentage between boys and girls.

Sex-stratified physical activity data are shown in Table 3.6. There was no evidence for differences in time spent in vigorous physical activity between boys and girls. However, there was significant difference on time spent in sedentary behaviour, with girls spending 2.6% more time in this risk behaviour in comparison to boys. Moderate to vigorous physical activity (MVPA) and overall physical activity was higher in boys than girls, with boys spending 23% and 11% more of their time in MVPA and total PA than girls respectively. These differences were not altered after adjusting the models for age and country. Time spent in MVPA was coded based on international physical activity guidelines recommendations, which suggest that 300 minutes per week of MVPA will contribute positively to our health. However, only 48 of the IDEFICS participants met this recommendation, and for this reason when the IDEFICS cohort was stratified on those who met 150 min of PA per week and those that did not meet this physical activity guideline, the result revealed that only 19.2% of the boys and 10.7% of girls spent more than 150 minutes of MVPA per week. Similarly, 10.9% and 11.1% for girls and boys respectively did not recorded vigorous physical activity.

Table 3-6 Time spent in sedentary behaviours and different intensities of physical activity in boys and girls.

	Boys		Girls		Unadjusted	Age-adjusted	Fully Adjusted
	n	Mean ± SD	n	Mean ± SD	*p*-value	*p*-value	*p*-value [Ŧ]
Sedentary time (min.day^{-1})	1063	606.8 ± 82.7	949	622.2 ± 80.0	<0.0001	<0.0001	<0.0001
MVPA (min.day^{-1})	1063	11.7 ± 9.4	949	9.5 ± 7.3	<0.0001	<0.0001	<0.0001
Vigorous activity (min.day^{-1})	1063	2.3 ± 2.8	949	2.5 ± 2.8	0.106	0.134	0.136
Overall Physical Activity (count.min^{-1})	1063	632.2 ± 169.5	949	568.8 ± 153.3	<0.0001	<0.0001	<0.0001

Data presented as unadjusted mean ± SD. P-values are given for unadjusted and adjusted data.
[Ŧ] Model adjusted for age, country and Actigraph wearing time.

Table 3.5 shows anthropometric characteristics in boys and girls stratified by age groups. These analyses aimed to reveal how anthropometric characteristics change with age. Understanding this relationship will provide a proxy on how obesity-related phenotypes change with maturation/growth process. There was a visible and significant trend of increased anthropometrics and obesity-related phenotypes with increasing age in both boys and girls for an unadjusted and adjusted analysis. Height, body mass, hip circumference and waist circumference showed a significant trend to increase with increasing age. However, for BMI, triceps and subscalpular skinfolds no differences were found between the age groups of 4, 5 and 6. From 7 year group onwards, the differences started to become significant in both sexes (see table 3.4). In order to verify whether these differences remained significant, a non-linear trend analysis was performed using Quadratic trend in STATA. However, these differences persisted, and therefore only linear trend was reported. Further adjustment for country did not alter these findings.

Anthropometrics and obesity-related phenotypes for boys and girls stratified by country are shown in table 3.6. Age showed significant differences between countries, with Belgium being the youngest group in both sexes and Hungary the oldest group across the countries for boys but not for girls. Height showed significant differences between countries, where Belgium had the shortest children in both sexes, Germany and Hungary the tallest for boys and Estonia for girls. Body mass index was significantly higher in Italian children compared to the remainder groups for both boys and girls, while Belgium reported a significant lower BMI compared to the other countries for boys but not for girls. Waist circumference showed a similar trend as BMI, with Italy and Cyprus showing a higher waist than the remainder countries for both sexes.

The lowest waist circumference was found in Belgium cohort for boys and in Germany for girls. Hips circumference showed the highest value in Italy and the lowest in Belgium for both boys and girls. Triceps and subscapular skinfold showed the highest values in Italy, while the lowest subscapular values were found in Belgium, for both sexes. The lowest triceps values were found in Sweden and Belgium for boys and Cyprus for girls. After adjustment for age, differences between countries remained similar.

Table 3-7 Anthropometric characteristic in boys and girls by age category.

	Age category <4	Age category 5	Age category 6	Age category 7	Age category 8	Age category <10	Unadjusted *p*-value	Country - adjusted p-value
Boys								
Height (cm)	99.0 ± 5.2 [#]	108.2 ± 5.0 [#]	114.6 ± 4.8 [#]	121.5 ± 5.8 [#]	127.4 ± 5.9 [#]	133.0 ± 6.1 [#]	<0.0001	<0.0001
Body Mass (kg)	15.7 ± 2.1 [#]	15.5 ± 2.5 [#]	20.6 ± 3.0 [#]	24.1 ± 4.8 [#]	27.2 ± 5.1 [#]	30.0 ± 5.6 [#]	<0.0001	<0.0001
BMI (kg.m^{-2})	15.9 ± 1.3 [8, 10]	15.8 ± 1.5 [8, 10]	15.6 ± 1.6 [8, 10]	16.3 ± 2.4 [10]	16.7 ± 2.4 [#]	16.9 ± 2.4 [#]	<0.0001	<0.0001
Waist (cm)	49.0 ± 3.1 [#]	51.4 ± 3.7 [#]	52.5 ± 3.7 [#]	55.3 ± 5.9 [#]	57.1 ± 5.8 [#]	59.0 ± 6.9 [#]	<0.0001	<0.0001
Hip (cm)	54.1 ± 3.5 [#]	57.6 ± 4.0 [#]	60.2 ± 4.5 [#]	64.5 ± 6.7 [#]	66.9 ± 6.0 [#]	69.4 ± 6.5 [#]	<0.0001	<0.0001
Subscapular (mm)	5.8 ± 1.2 [8, 10]	5.9 ± 1.5 [8, 10]	5.7 ± 1.6 [8, 10]	6.4 ± 2.8 [6, 10]	6.5 ± 2.7 [4,5,6,10]	7.2 ± 3.7 [#]	<0.0001	<0.0001
Triceps (mm)	9.8 ± 2.2 [8, 10]	9.9 ± 2.4 [8, 10]	9.3 ± 2.4 [8, 10]	9.9 ± 3.6 [8, 10]	10.6 ± 3.8 [#]	11.4 ± 4.3 [#]	<0.0001	<0.0001
Girls								
Height (cm)	98.7 ± 5.8 [#]	106.2 ± 5.0 [#]	113.5 ± 5.0 [#]	119.6 ± 5.6 [#]	126.5 ± 5.6 [#]	131.8 ± 5.1 [#]	<0.0001	<0.0001
Body Mass (kg)	15.5 ± 2.3 [#]	17.6 ± 2.4 [#]	20.4 ± 3.1 [#]	23.2 ± 4.1 [#]	26.5 ± 5.0 [#]	30.4 ± 6.5 [#]	<0.0001	<0.0001
BMI (kg.m^{-2})	15.8 ± 12.3 [8,10]	15.6 ± 0.5 [8,10]	15.7 ± 1.7 [8,10]	16.2 ± 2.1 [9]	16.5 ± 2.4 [4,5,6,10]	17.4 ± 3.0 [#]	<0.0001	<0.0001
Waist (cm)	48.9 ± 3.5 [#]	50.4 ± 3.7 [#]	52.6 ± 4.5 [#]	54.5 ± 5.7 [#]	56.2 ± 6.2 [#]	58.9 ± 7.3 [#]	<0.0001	<0.0001
Hip (cm)	54.7 ± 3.9 [#]	57.8 ± 3.9 [#]	60.8 ± 4.5 [#]	64.2 ± 5.7 [#]	66.9 ± 6.2 [#]	71.1 ± 7.3 [#]	<0.0001	<0.0001
Subscapular (mm)	6.5 ± 1.5 [7,8,10]	6.3 ± 0.6 [7,8,10]	6.7 ± 2.1 [8,10]	7.4 ± 3.1 [4,5,10]	7.7 ± 3.5 [4,5,6,10]	8.9 ± 4.9 [#]	<0.0001	<0.0001
Triceps (mm)	10.8 ± 2.5 [8,10]	11.2 ± 2.8 [8,10]	11.0 ± 2.9 [8,10]	11.7 ± 3.5 [4,5,10]	12.1 ± 4.1 [4,5,6,10]	13.9 ± 5.2 [#]	<0.0001	<0.0001

Data presented as unadjusted mean ± SD. P-values for differences between ages are given for unadjusted and country-adjusted data. Bonferroni post-hoc test was used to detect significant differences between age groups ($p<0.05$). These differences were denoted as numbers ([4, 5, 6, 7, 8, 10]) which indicate differences between specific age groups, and [#] which indicates a difference amongst all age groups. For trend analysis, the age groups were coded as ordinal numbers from 0 to 5, and fitted in a simple and multiple regression analysis, for unadjusted and adjusted analysis, respectively.

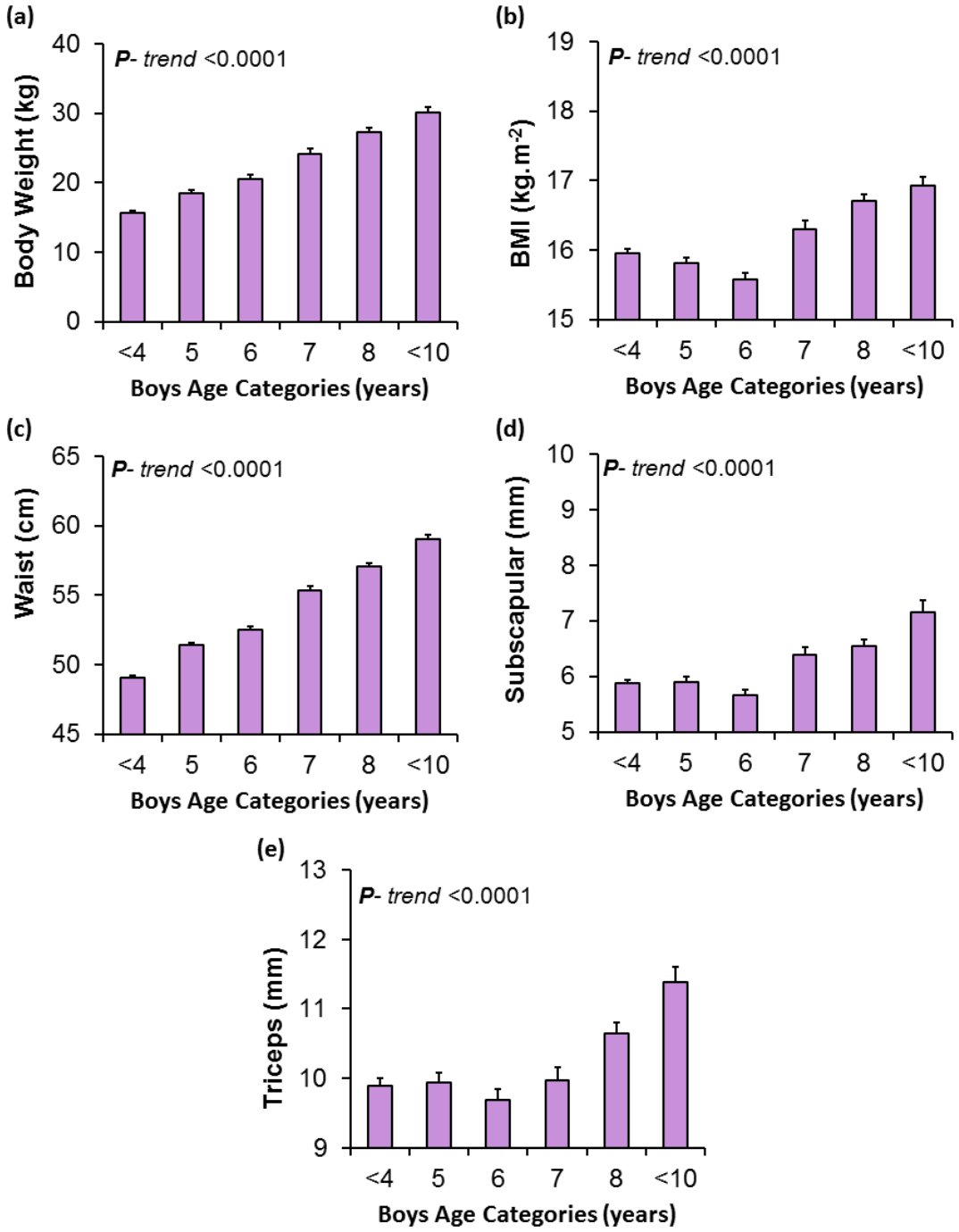


Figure 3.2 Anthropometric characteristics in boys by age categories.

Data presented as unadjusted mean ± SEM. P-values for differences between age categories in boys were calculated for unadjusted data.

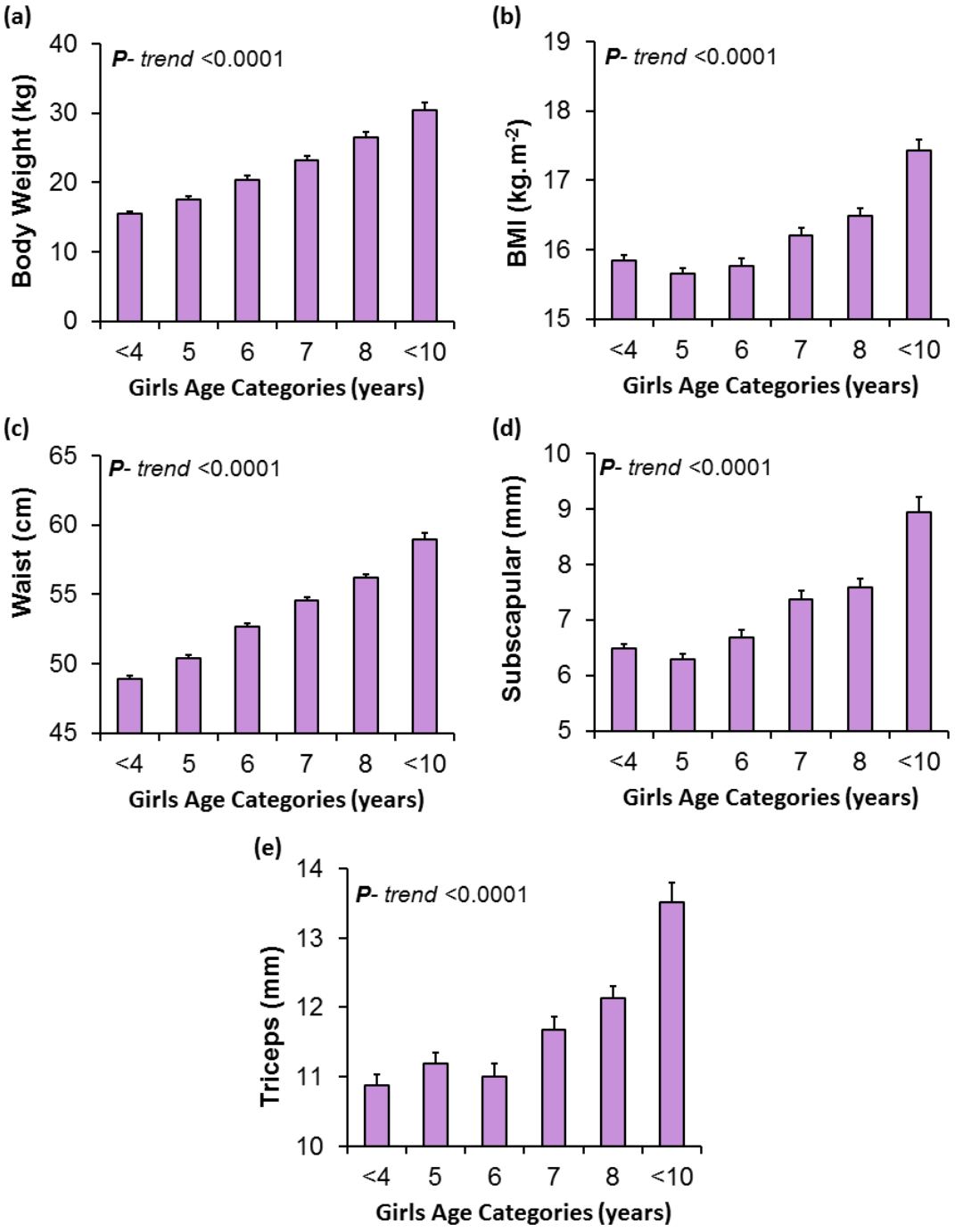


Figure 3.3 Anthropometric characteristic in girls by age categories.

Data presented as unadjusted mean ± SEM. P-values for differences between age categories in girls were calculated for unadjusted data.

Physical activity patterns by age categories in boys and girls were described in table 3.7. Of a total sample of n=4407 that was recruited, only n=2012 had physical activity data, number for each specific age and sex are given in table 3.6. Unadjusted data shows a visible trend to increase time spent in sedentary activities with increasing age for both sexes (p<0.0001). Significant differences on sedentary time were found between the ages of 4-5 years and ≥8. Girls showed significant differences between the age of 4-5 and ≥6 years old in physical activity patterns. A similar trend to increase MVPA with increasing age was found in both boys and girls

($p<0.0001$). Both sexes spend more time in MVPA when they are older (>6 years) compared to those younger than 6 years. Time spent in vigorous activity increased significantly with increasing age for boys ($p<0.001$) and girls ($p<0.0001$). Time spent in overall physical activity shows a decreasing trend as age in boys increases ($p<0.0002$) but no significant trend was observed for girls ($p=0.292$). After adjusting the models for several confounding factors such as country and accelerometer wearing time, the previous result remained the same.

Table 3.8 shows physical activity patterns stratified by country. Time spent in sedentary behaviours was significantly higher in Cyprus and Italy, while countries with less time spent in this risk behaviours were Belgium, Germany and Hungary, and this differences were observed in both boys and girls. Time spent in moderate to vigorous physical activity was significantly lower in Cyprus and Italy for both sexes, whereas Germany and Spain for boys and Estonia, Germany and Sweden for girls showed the highest time spent in MVPA compared to the other countries. No differences were observed for time spent in vigorous physical activity in boys; however girls showed that in Germany, Estonia, Sweden and Hungary spent more time in this intensity compared to Spain, Italy and Cyprus. Overall time spent in physical activity was lower in Cyprus and Italy and higher in Sweden and Germany for both sexes.

Table 3-8 Anthropometric characteristic in boys and girls by country.

	Italy (1)	Estonia (2)	Cyprus (3)	Belgium (4)	Sweden (5)	Germany (6)	Hungary (7)	Spain (8)	Unadjusted *p*-value	Age - adjusted p-value
Boys										
Age	6.1 ± 1.7 [4]	5.9 ± 2.1 [7]	6.1 ± 1.3 [4]	5.6 ± 1.7 [1,3,6,7]	5.8 ± 2.1 [6,7]	6.2 ± 1.7 [4,5]	6.5 ± 1.7 [2,4,5,8]	5.9 ± 1.7 [7]	<0.0001	-
Height (cm)	117.6 ±12.3	118.5±14.9 [4]	118.0± 9.3	114.6 ± 11.9 [2,6,7]	117.1 ± 15.3 [7]	120.1 ± 12.3 [4,8]	120.9 ± 12.5 [4,5,8]	116.6 ± 13.1 [6,7]	<0.0001	<0.0001
Body Mass (kg)	24.7 ± 7.5 [4,5,8]	23.3 ± 7.3 [4]	23.1 ± 5.6 [4]	20.7 ± 4.8 [#]	22.3 ± 6.6 [1,4,7]	23.4 ± 6.0 [4]	24.1 ± 6.8 [4,5]	22.7 ± 6.5 [1,4]	<0.0001	<0.0001
BMI (kg.m^{-2})	17.6 ± 2.5 [#]	16.2 ± 1.9 [1,4]	16.3 ± 2.3 [1,4]	15.5 ± 1.4 [#]	15.9 ± 1.8 [1,4]	16.0 ± 1.8 [1,4]	16.2 ± 2.2 [1,4]	16.4 ± 1.9 [1,4]	<0.0001	<0.0001
Waist (cm)	56.7 ± 8.0 [#]	54.2 ± 6.1 [1,3,4,6]	55.9 ± 6.3 [#]	52.4 ± 4.2 [1,2,3,7,8]	53.5 ± 5.4 [1,3,7]	52.6 ± 5.3 [1,2,3,7,8]	55.3 ± 6.6 [1,4,5,6]	54.1 ± 5.9 [1,3,4,6]	<0.0001	<0.0001
Hip (cm)	66.3± 8.7 [#]	61.9± 7.6 [1,3,4,7]	63.4 ± 6.9 [1,2,4,5,6]	59.5 ± 5.9 [#]	61.3 ± 7.5 [1,3,4,7]	62.0 ± 6.8 [1,3,4,7]	63.4 ± 7.9 [1,2,4,5,6]	62.4 ± 7.7 [1,2,4,5,6]	<0.0001	<0.0001
Subscapular (mm)	7.6 ± 3.5 [#]	6.1 ± 2.5 [1]	6.8 ± 2.7 [1,4]	5.5 ± 1.3 [1,3]	5.8 ± 1.8 [1,8]	6.1 ± 1.9 [1]	6.2 ± 2.8 [1,4]	6.6 ± 2.6 [1,4,5]	<0.0001	<0.0001
Triceps (mm)	11.1 ± 4.2 [4,5,7]	10.5 ± 3.0 [5]	10.3 ± 3.5	9.6 ± 2.4 [1]	9.4 ± 2.7 [1,2,6,7]	10.5 ± 3.3 [5]	10.1 ± 3.4 [1]	10.5 ± 3.7 [5]	<0.0001	<0.0001
Girls										
Age	6.3 ± 1.7 [4]	6.1 ± 2.0 [4]	6.2 ± 1.2 [4]	5.6 ± 1.6 [1,2,3,7]	6.0 ± 1.9	6.0 ± 1.7	6.2 ± 1.8 [4]	5.9 ± 1.7	<0.0001	-
Height (cm)	117.8 ±12.7 [4]	118.7±14.3 [4,8]	117.5 ± 9.4 [4]	113.9 ± 11.6 [#]	117.4 ± 13.9 [4]	117.4 ± 12.4 [4]	117.9 ± 12.8 [4]	114.9 ± 12.8 [2,4]	<0.0001	<0.0001
Body Mass (kg)	25.5 ± 8.5 [#]	23.2 ± 7.1 [1,4]	22.5 ± 5.5 [1,4]	20.6 ± 4.8 [1,2,3,7]	22.3 ± 6.0 [1]	22.4 ± 6.2 [1]	22.8 ± 6.8 [1]	22.2 ± 6.5 [1]	<0.0001	<0.0001
BMI (kg.m^{-2})	17.8 ± 2.8 [#]	16.1 ± 2.1 [1]	15.9 ± 2.0 [1]	15.7 ± 1.7 [1]	15.9 ± 1.8 [1]	16.0 ± 1.8 [1]	16.0 ± 2.2 [1]	16.5 ± 2.0 [1]	<0.0001	<0.0001
Waist (cm)	57.0 ± 8.5 [#]	53.2 ± 5.8 [1,3]	56.1 ± 6.5 [#]	52.2 ± 4.5 [1,3]	53.0 ± 5.2 [1,3]	51.9 ± 5.4 [1,3]	54.1 ± 6.4 [1,3]	53.5 ± 6.1 [1,3]	<0.0001	<0.0001
Hip (cm)	67.7± 9.3 [#]	63.2± 7.5 [1,4]	63.5 ± 6.9 [1,4]	60.3 ± 5.9 [#]	61.9 ± 7.0 [1,4]	61.8 ± 6.9 [1,4]	62.8 ± 7.8 [1,4]	62.7 ± 7.9 [1,4]	<0.0001	<0.0001
Subscapular (mm)	9.6 ± 4.8 [#]	6.7 ± 2.9 [1,8]	7.2 ± 2.9 [1,8]	6.5 ± 2.2 [1,8]	6.6 ± 2.5 [1,8]	7.0 ± 2.4 [1,8]	6.9 ± 2.9 [1,8]	7.6 ± 3.1 [#]	<0.0001	<0.0001
Triceps (mm)	13.4 ± 5.1 [#]	11.8 ± 3.6 [1]	10.8 ± 3.1 [1,6,8]	11.4 ± 3.1 [1,6]	11.0 ± 3.2 [1,6,8]	12.6 ± 3.7 [1,3,4,5,7]	11.2 ± 3.5 [1,6,8]	12.3 ± 4.1 [1,3,5,7]	<0.0001	<0.0001

Data presented as unadjusted mean ± SD. P-values for differences between countries are given for unadjusted and age-adjusted data. Bonferroni post-hoc test was used to detect significant differences between countries ($p<0.05$), these differences were denoted as numbers ([1,2,3,4, 5, 6, 7, 8]) which indicate differences between specific countries and [#] indicates differences amongst all countries.

Table 3-9 Objectively measured physical activity patterns in boys and girls by age category.

	Age category <4	Age category 5	Age category 6	Age category 7	Age category 8	Age category <10	Unadjusted *p*-value	Adjusted p-value[T]
Boys (n=1063)	163	162	105	153	282	198		
Sedentary time (min.day^{-1})	586.9 ± 77.8 [8,10]	593.2 ± 79.8 [8,10]	610.1 ± 95.1	607.5 ± 84.3	615.4 ± 80.2 [4,5]	619.9 ± 80.4 [4,5]	<0.0001	0.0002
MVPA (min.day^{-1}	6.9 ± 5.5 [#]	10.3 ± 9.1 [4,8,10]	10.0 ± 9.0 [4,8,10]	12.5 ± 9.5 [4]	13.8 ± 9.9 [4,5,6]	14.2 ± 9.8 [4,5,6]	<0.0001	<0.0001
Vigorous activity (min.day^{-1})	1.6 ± 1.8 [8,10]	2.3 ± 3.1 [8,10]	2.1 ± 2.6	2.2 ± 2.4	2.6 ± 3.2 [4]	2.7 ± 3.1 [4]	0.001	0.001
Overall Physical Activity (count.min^{-1})	612.6 ± 155.6 [5]	676.1 ± 168.1 [4,8,10]	654.3 ± 184.9	639.3 ± 161.6	623.2 ± 162.1 [5]	607.8 ± 182.5 [5]	0.0002	0.0005
Girls (n=949)	140	129	92	144	272	172		
Sedentary time (min.day^{-1})	596.7 ± 71.9 [6,7,8,10]	598.5 ± 87.4 [6,7,8,10]	631.1 ± 91.5 [4,5]	628.5 ± 72.1 [4,5]	628.9 ± 74.9 [4,5]	639.8 ± 80.2 [4,5]	<0.0001	<0.0001
MVPA (min.day^{-1})	5.6 ± 4.6 [7,8,10]	7.3 ± 5.6 [7,8,10]	7.7 ± 4.7 [8,10]	9.8 ± 7.4 [4,5]	11.7 ± 7.7 [4,5,6]	11.4 ± 8.8 [4,5,6]	<0.0001	<0.0001
Vigorous activity (min.day^{-1})	1.7 ± 2.6 [8,10]	1.8 ± 2.1 [8,10]	1.9 ± 1.8 [8,10]	2.5 ± 2.6 [4,5,6]	3.1 ± 3.1 [4,5,6]	3.1 ± 3.3 [4,5,6]	<0.0001	<0.0001
Overall Physical Activity (count.min^{-1})	564.2 ± 132.9	577.2 ± 132.9	598.3 ± 150.9	587.9 ± 177.3	562.3 ± 144.8	544.9 ± 171.8	0.292	0.307

Data presented as unadjusted mean ± SD. P-values for differences between ages are given for unadjusted and adjusted data. Bonferroni post-hoc test was used to detect significant differences between age groups ($p<0.05$), these differences were denoted as numbers ([4, 5, 6, 7, 8, 10]) which indicate differences between specific age groups and [#] indicates differences amongst all age groups. [T] Model adjusted for country and Actigraph wearing time.

Table 3-10 Objectively measured physical activity patterns in boys and girls by country.

	Italy (1)	Estonia (2)	Cyprus (3)	Belgium (4)	Sweden (5)	Germany (6)	Hungary (7)	Spain (8)	Unadjusted p-value	Adjusted p-value
Boys (n=1063)	125	181	60	75	98	177	88	259		
Sedentary time (min.day^{-1})	644.8 ± 87.3 #	601.9 ± 76.5 1,3,6,7	704.5 ± 116.2 #	587.3 ± 59.5 1,3,5	624.1 ± 72.9 3,4,6,7	564.1 ± 73.4 1,2,3,5,8	568.4 ± 75.9 1,2,3,5,8	610.5 ± 61.2 1,3,6,7	<0.0001	<0.0001
MVPA (min.day^{-1})	9.8 ± 8.5 6	11.7 ± 10.3	8.2 ± 5.8 6,8	11.3 ± 7.1	12.5 ± 8.5	14.2 ± 11.5 1,3,7	9.9 ± 7.4 6	12.4 ± 9.4 3	<0.0001	<0.0001
Vigorous activity (min.day^{-1})	1.9 ± 2.5	2.7 ± 3.8	1.8 ± 1.8	2.1 ± 1.9	2.4 ± 2.4	3.0 ± 3.5	1.9 ± 1.8	2.1 ± 2.5	0.207	0.339
Overall Physical Activity (count.min^{-1})	552.2 ± 178.3 2,4,5,6,8	635.9 ± 153.5 1,3,6	540.9 ± 126.6 2,4,5,6,8	649.8 ± 134.8 1,3	665.5 ± 164.5 1,3,7	703.1 ± 194.1 1,2,3,7,8	577.6 ± 161.7 5,6,8	641.6 ± 151.1 1,3,6,7	<0.0001	<0.0001
Girls (n=949)	120	183	57	79	85	146	66	213		
Sedentary time (min.day^{-1})	674.4 ± 68.0 #	605.5 ± 72.4 1,3,6,8	729.2 ± 117.9 #	591.1 ± 57.9 1,3,5,8	625.3 ± 65.9 1,3,4,6,7	581.6 ± 68.1 1,2,3,5,8	581.8 ± 61.2 1,,3,5,8	628.9 ± 60.5 1,2,3,4,6,7	<0.0001	<0.0001
MVPA (min.day^{-1})	6.8 ± 5.8 2,5,6	11.3 ± 8.4 1,8	6.1 ± 4.7 2,5,6	8.5 ± 5.5	11.5 ± 7.6 1,3,8	11.3 ±7.8 1,3,8	9.6 ± 6.9	8.5 ± 7.1 2,5,6	<0.0001	<0.0001
Vigorous activity (min.day^{-1})	1.7 ± 2.3 2,5,6	3.2 ± 3.3 1,3,8	1.7 ± 2.1 2,5,6	2.2 ± 2.1	3.4 ± 3.5 1,3,8	3.0 ± 2.8 1,3,8	2.8 ± 3.3	1.9 ± 2.3 2,5,6	<0.0001	<0.0001
Overall Physical Activity (count.min^{-1})	475.6 ± 179.9 2,4,5,6,8	593.4 ± 128.4 1,3,7	487.4 ± 148.5 2,4,5,6	573.7 ± 111.8 1,3,5,6	647.2 ± 168.7 1,3,4,7,8	641.6 ± 151.6 1,3,4,7,8	521.5 ± 124.8 2,5,6	553.8 ± 127.5 1,5,6	<0.0001	<0.0001

Data presented as unadjusted mean ± SD. P-values for differences between countries are given for unadjusted and adjusted data. Bonferroni post-hoc test was used to detect significant differences between countries ($p<0.05$), these differences were denoted as numbers ($^{4, 5, 6, 7, 8, 9}$) which indicate differences between specific countries and # indicates differences amongst all countries. ᵀ Model adjusted for age and Actigraph wearing time.

3.6 Discussion

The main findings of this chapter were: (a) significant differences in height, waist and hip circumference, subscapular and triceps skinfolds between boys and girls (b) there was strong evidence for differences in time spent in sedentary, moderate to vigorous physical activity (MVPA) and overall physical activity between boys and girls, with girls spending more time in sedentary behaviours; (c) when comparing the obesity phenotypes by country, Italy had the children with the highest body mass index (BMI), waist and hip circumferences, subscapular and triceps skinfolds for boys and girls; (d) PA trends in stratified age groups showed a visible trend where an increase in time spent in sedentary and MVPA activities was observed with increasing age for both sexes. However, vigorous physical activity time increased significantly with increasing age, and time spent in overall physical activity showed a trend to decrease with increasing age in boys but not girls; (e) physical activity patterns stratified by country showed increased sedentary behaviour, lower MVPA and time spent in overall PA in Italy and Cyprus and less time spent in this sedentary behaviours were observed for Belgium, Germany and Hungary.

The finding that there were significant differences in height, waist and hip circumference, subscapular and triceps skinfolds between boys and girls, is on par with previous work in this field, which indicates higher body fat in females compared to males (Shaw et al. 2007). One possible hypothesis is that gender differences may be lost at the extremes of obesity. In addition, this chapter revealed strong evidence of variation on time spent in sedentary, moderate to vigorous physical activity (MVPA) and overall physical activity between boys and girls, with girls spending more time in sedentary behaviours. Again, this finding confirms previous data from the Health Survey for England which examined secular trends in physical activity participation and health behaviours and identified that at all ages and stages of development boys are more active than girls (UKNS 2006). In the present study, a significant trend of increased anthropometrics and obesity-related phenotypes with increasing age was observed when comparing age groups. Although this trend is well known, as maturation and growth play an important role in increasing anthropometric and adiposity-related phenotype with increasing age. This analysis was performed in order to establish the increase pattern in these obesity-related markers with increasing age. These analyses revealed that although weight and waist circumference increase linearly with increasing age, other obesity related markers such as BMI, and skinfolds thickness do not follow the same trend. These markers showed lesser flat trend before the age of 7, however after the age of 7 these markers considerably increased in both boys and girls.

Previous research in healthy children and adolescents found girls to have on average 3.8% more fat than boys at the age of 5 years, increasing to 12.9% by the age of 18 years (Shaw et al. 2007), suggesting a role of pubertal stage in body fat. Adding to this, puberty is known to affect body composition, with increases in all aspects of body composition (fat mass, lean mass etc.) observed during the period of pubertal growth and development (Siervogel et al. 2003). Therefore, the wide age range of this study influences body composition parameters in a

significant way. However, as mentioned previously, when comparing the obesity phenotypes by country, Italy had the children with the highest body mass index (BMI), waist and hip circumferences, subscapular and triceps skinfolds for boys and girls compared with all the other countries. This difference in measurements in Italy could be related to the area from which the data was collected. In this scenario, it could be the case that there were more overweight children in the area where the field work in Italy was conducted and therefore, the increased body composition measurements may not be directly related to the country.

Engaging in regular physical activity is widely accepted as an effective preventative measure for a variety of health risk factors across all age, gender, ethnic and socioeconomic subgroups (Janssen and Leblanc 2010). However, across all age groups, levels of physical activity remain low (Troiano et al. 2008), and obesity rates continue to rise collectively threatening the persistent increase in life expectancy enjoyed over the past century and efforts to counteract the inactivity and obesity crisis (Olshansky et al. 2005). This inactivity crisis is especially important in the paediatric population as recent data from international Survey (Colley et al. 2011), suggest that only 7% of children and youth aged 4-19 years old participate in at least 60 minutes of moderate- to vigorous-intensity physical activity per day, thus meeting the current physical activity guidelines from Canada (Tremblay et al. 2011), U.S., U.K, Australia and World Health Organization (Guthold et al. 2010). The IDEFICS cohort provided a clearer and more updated picture of sedentary behaviours in European children, estimating that just 4% of children with ages below 10 years old meet the international recommendation of 60 minutes of MVPA per day. However, even for those children who meet current guidelines, there remains 23 hours per day for school, sleep, work, and discretionary time. Several sources report that children and youth spend the majority of their discretionary time engaging in sedentary pursuits (e.g. watching television (TV) or playing video games) (Guthold et al. 2010). In average, children are spending an average of 8.6 hours per day, or 62% of their waking hours being sedentary (Guthold et al. 2010). Similar trends are being reported in the U.S. where children spend an average of 6-8 hours per day being sedentary (Guthold et al. 2010). However, European children spend an average of 11 hours per day being sedentary. These indicate that 3 extra hours are spent by European children on this risk behaviour compared to children in the US. This has an important health implication as accumulating evidence show that, independent of physical activity levels, sedentary behaviours are associated with increased risk of cardio-metabolic disease, all-cause mortality, and a variety of physiological and psychological problems (Tremblay et al. 2010).

In addition, the current study found that PA trends in stratified age groups showed a visible trend revealing an increase in time spent in sedentary and MVPA activities with increasing age for both sexes. However, vigorous physical activity time increased significantly with increasing age and time spent in overall physical activity showed decreasing trend with increasing age in

boys but not girls. This could be explained by the fact that older children may choose more sedentary activities outside of school.

Interestingly, when physical activity patterns were stratified by country, this showed an increase in sedentary behaviour, lower MVPA and time spent in overall PA in Italy and Cyprus and less time spent in these sedentary behaviours was observed for Belgium, Germany and Hungary. However, it is important to be conscious when interpreting country effects as many factors could be involved in the finding, involving national and local systems, such as differential influences within schools, and physical activity environments available. On the contrary, Italy and Cyprus (table 3.7) are countries close to each other and one could hypothesize the prevalence of a more sedentary lifestyle in Mediterranean countries.

Some of the main strengths of the present study is the large sample recruited across eight European countries. In addition, another strength of the study is the use of precisely standardized phenotypic measurements within the eight European countries participating in the survey. In fact, all measurements were conducted according to detailed standard operation procedures. However, despite the strengths of this study mentioned above, there are a number of limitations. Firstly, it cannot be ruled out the possibility that our results may be marginally influenced by population stratification, since no neutral markers were genotyped. Secondly, the study participants were not nationally representative of the eight European countries involved in the IDEFICS study, as this was never the study's objective. A randomly selected subgroup from the whole IDEFICS cohort which had age-, sex- and country-matched of white European children was extracted. In our analysis, however, all data were further adjusted for age, sex and country. Furthermore, the existence of multiple sets of PA cut points for children and adolescents is a potential limitation when estimating physical activity behaviour of youths. To date, at least five sets of youth-specific Actigraph cut points have been independently developed and published in the peer-reviewed scientific literature (Puyau et al. 2002; Treuth et al. 2004; Freedson et al. 2005; Mattocks et al. 2007; Evenson et al. 2008). The method used to derive these cut points varied considerably from study to study. For example, some methods use age and sex specific cut points and others do not; some methods measure PA based on fixed (i.e. treadmill) or free-play and land others on lifestyle activities. In this study, the cut points used were more conservative than cut-off points derived by a recent study (Evenson et al. 2008) and thus this could explain why children presented a low record of time spent in moderate and vigorous activity (Evenson et al. 2008). This again is related to methodological issues of usage of non-uniform PA cut points for specific populations and it leads to possible inaccuracies when interpreting the PA data collected from the children in those specific populations. In a recent validation study, Mackintosh et.al proposed that cut-points of <=372, >2160 and >4806 counts per minute represented sedentary, moderate and vigorous intensity thresholds respectively, and provide the optimal balance between the related needs for sensitivity (accurately detecting activity) and specificity (limiting misclassification of the activity) (Mackintosh et al. 2012). In addition, another limitation could be considered that even though the recruitment was

performed by selecting random samples with minimum obesity-related phenotypes available, this was only balanced by sex, allowing a maximum distribution of 40/60 percent within genders. However, the recruitment was not strictly stratified or balanced by other factors such as country, or age groups. This could bring some confounder effect into the interpretation of the data. Furthermore, unadjusted and fully adjusted models were given when differences in physical activity or obesity-related markers between boys and girls were examined.

In conclusion, the present chapter reveals that age is an important factor when studying childhood obesity related traits which change in a significant way with increasing age, in both boys and girls. These findings also highlight the fact that environmental and lifestyle effects such as PA on childhood obesity differ between the two sexes and among age groups. There is also a conceivable influence of the country of origin on the prevalence of childhood obesity; however, further studies will provide deeper understanding into the mechanisms and other confounding factors involved, underpinning this effect. In addition, developing a better understanding regarding the influences of schools and community, will aid in more effective implementation of lifestyle strategies in the future in order to reduce the prevalence of childhood obesity in different countries.

4 Association of obesity patterns and physical activity Lifestyle Patterns in the IDEFICS population

4.1 Introduction

It is well recognized that physical activity has a protective role in the prevention and treatment of obesity and other related diseases in very young children (Reilly 2008). However, the environment appears to have a strong impact on the development of obesity, thus investigating this area more deeply will aid in better understanding the mechanisms and etiologies of obesity, and will also assist in the development of better intervention and prevention studies.

Among other environmental factors, sedentary behaviour and decreased time spent in physical activity was found to be associated with increased body fat in young children (Ekelund et al. 2004). One important factor associated with sedentary behaviour in children, can be the decreased time spent in activities outdoors (Cleland et al. 2008). Evidence show that young children aging from three to five years, spend around 80% of their time in activities classified as sedentary or light physical activity (Reilly et al. 2004). In addition, studies support that sedentary behaviour in children, particularly television viewing, is associated with adiposity-related outcomes (Reilly 2008); and increased physical activity is inversely associated with metabolic syndrome (Brage et al. 2004), suggesting a protective role of PA. Another interesting aspect of the relationship between PA and obesity is the identification of the age group at which PA decline appears. Levels of PA during childhood are expected to be stable with age, and appear to decline during adolescence. However, a recent longitudinal study, reported low levels of physical activity and increasing time in sedentary behaviour before adolescence in both boys and girls (Basterfield et al. 2010). This finding suggests that intervention strategies to promote PA and prevent the increased time spent in sedentary activities should start before adolescence.

In order to better understand the relationship between physical activity and heath in general, particularly obesity, it is important to use valid and reliable instruments for assessing PA in children and adolescents. Using questionnaires to assess physical activity in children is not considered a very accurate method, as children often over or under estimate their PA habits. Thus far, accelerometry is considered the most efficient way to assess PA since it is a method which provides an objective measure of habitual activity, it does not rely on self-report and is superior to pedometers as it provides intensity as well as frequency (Mathie et al. 2004). In order to quantify intensity, accelerometers measure the change in velocity over time (acceleration) ($m.s^{-2}$) (Freedson et al. 2005). Accelerometers can be uniaxial, measuring movement in the vertical plane, biaxial or triaxial and use cut offs to determine levels of physical activity such as

Pate's and Sirard's cut off points (Sirard and Pate 2001; Pate et al. 2006). Accelerometers have advantages over more traditional methods of measuring PA such as: the avoidance of bias, greater confidence in the amount of activity and sedentary behaviour measured. Furthermore, they also provide improved ability to relate variation in physical activity and sedentary behaviour to variation in health outcomes such as obesity (Reilly et al. 2008). In a study designed to develop valid tools to assess eating and physical activity patterns among middle school children, it was reported that accelerometers were acceptable to most students, however, careful monitor of compliance was essential in order to ensure that devices were worn properly and regularly (Van Coevering et al. 2005). This highlights the fact that although accelerometers are the most objective measure to assess PA in children, it is important to monitor compliance in correctly using the device. It is important to mention that IDEFICS is one of the largest studies conducted which provides accelerometer data in order to assess physical activity patterns and behaviours in young European children.

The aims of this chapter are:

(a) To examine the association of physical activity and inactivity on adiposity-related traits in European children.

(b) To determine whether the influences of adiposity, physical activity and inactivity on childhood obesity differ between age or sex in European children.

4.2 Research Design and Methods

4.2.1 Participants and Data Collection

The sample studied in this section includes only individuals who were genotyped for the *FTO* gene and have physical activity data (n=2012). All anthropometric measurements were conducted in the participating schools of the study. Weight, height, waist and hip circumferences and skinfolds at four sites (biceps, triceps, subscapular, and suprailiac) were measured following standard protocols mentioned in detail within sections 2.2.

Physical activity patterns and sedentary behaviour were objectively measured using Actigraphs and Actitrainer (ActiGraph GT1M, ActiTrainer, Polar Heart Rate monitor strap). Prior to testing, each accelerometer was tested, fully charged and had its memory erased. Children with any physical handicap or constrained movement were excluded from this measurement. Each child had to wear a device during waking hours for three consecutive full days. Ideally, two of the days were weekdays and the other one weekend day. Parents were instructed to fill out an activity diary for the days the child would wear the accelerometer. This diary was used as a reference to monitor the children's compliance to wearing the device properly and for the time instructed; this data was not used in the analysis per se. Parents also received an instruction document on how to use the device and were advised to remove the device from the children

prior to bathing, swimming and showering as the device is not waterproof. The activity monitor had to be attached around the waist on the right hip side underneath the clothes, immediately when the child got up and only removed when it was time to go to bed at night. The ActiTrainer was also worn around the waist on the right hip side and underneath the child's clothes, along with the heart rate monitor strap which was placed around the chest.

Physical activity patterns were calculated based on Sirard's physical activity cut-off points for children (Sirard 2005). Average time spent in sedentary behaviour was calculated using a cut-off point of <398 counts per 15 second, average time spent in light intensity physical activities were estimated using a cut-off ranging between >398 and <890 counts per 15 seconds), average time spent in moderate intensity physical activity was estimated using cut-off point ranging between >890 and <1254 count per 15 seconds) and average time spent in vigorous intensity physical activity was estimated using a cut-off point above 1254 counts per 15 seconds. An average overall physical activity variable was derived from adding the sum of average time spent in light, moderate and vigorous intensities physical activities during the three measurement days (this variable was expressed in count per minute per day as different intensities were sum up). Moderate to vigorous physical activity (MVPA) was derived by adding the sum of minutes spent in moderate and vigorous activities (this variable was expressed in minutes per day, as international guidelines on recommended level of physical activities for this specific intensity domain are expressed in minutes per day). Neither overall PA nor MVPA include time spent in sedentary behaviors. Valid accelerometer worn time was always accounted for when time spent in different physical activity intensity was studied, in order to have more accurate activity or inactivity patterns.

4.3 Statistical analysis

Statistical analysis was performed using STATA (version 11; Statacorp, TX, USA) and Statistica (version 8.0, StataSoft, Tulsa, USA). All continuous variables were checked for a normal distribution using Anderson-Darling test. Categorical variables such as sex (0=boys; 1=girls) and country (Italy, Estonia, Cyprus, Belgium, Sweden, Germany, Hungary, Spain) were coded. Physical activity variables were derived using Sirard's criteria. These measurements were available for n=2012 participants. Age and sex specific tertiles were derived for sedentary behavior and physical activity. Tertile of time spent in sedentary behaviour was estimated. Moderate to vigorous physical activity (MVPA) was derived from the sum of time spent in moderate and vigorous physical activity). Tertile of overall physical activity was estimated from the sum of time spent in light, moderate and vigorous physical activity. All the physical activity tertiles are presented in Table 4.1.

Simple linear regression was used to determine the relation between physical activity variables, birth weight and age with adiposity-related outcomes. Multiple regressions were used to measure the independent association of the explanatory factor and the adiposity-related outcome after adjusting for covariates (age, sex, country and accelerometer wearing times). The regression

outputs were presented as regression coefficients and their 95%CI and also as standardized beta coefficient with their standard error, which indicate an SD change in the outcome for one SD change in the explanatory factor. General Linear Model statistic framework was used to assess whether the relationship between physical activity patterns and adiposity factors differs by age and sex. In order to assess the interaction, age and sex were fitted into the model as categorical factors. When no evidence of significant interaction was found the age and sex factor were removed from the interaction and fitted as an adjusting factor into the model. Tertiles of physical activity-related variables were derived and denoted as 0=lower, 1=middle and 2=higher time spent in sedentary behaviour, MVPA and vigorous PA.

Odd ratio and their 95%CI were assessed using logistic regression for binary outcomes. Obesity categories derived from BMI using Cole definition (Cole et al. 2000), and adjusting variables (age, sex and country) were fitted into the model to adjust for potential confounding factors. A *p*-value <0.05 was accepted as significant.

Table 4-1 Description of age and sex specific tertiles of Physical activity.

		Boys				Girls			
Age groups	Physical Activity Domain	N	Low (<)	Middle	Upper (>)	N	Low (<)	Middle	Upper (>)
4	Sedentary	163	550.0	550.0 to 613.0	613.0	140	564.5	564.5 to 616.3	616.3
	MVPA	163	3.7	3.7 to 7.8	7.8	140	3.3	3.3 to 6.0	6.0
	Overall PA	163	532.7	532.7 to 669.5	669.5	140	500.5	500.5 to 600.2	600.2
5	Sedentary	162	562.3	562.3 to 617.6	617.6	129	566.0	566.0 to 624.0	624.0
	MVPA	162	4.8	4.8 to 11.0	11.0	129	4.3	4.3 to 7.4	7.4
	Overall PA	162	583.7	583.7 to 733.9	733.9	129	530.7	530.7 to 620.9	620.9
6	Sedentary	105	573.7	573.7 to 626.2	626.2	92	581.0	581.0 to 657.5	657.5
	MVPA	105	5.3	5.3 to 10.7	10.7	92	5.0	5.0 to 9.3	9.3
	Overall PA	105	564.1	564.1 to 704.2	704.2	92	527.0	527.0 to 640.5	640.5
7	Sedentary	153	573.0	573.0 to 635.0	635.0	144	604.5	604.5 to 644.7	644.7
	MVPA	153	7.6	7.6 to 13.9	13.9	144	6.0	6.0 to 10.1	10.1
	Overall PA	153	562.8	562.8 to 698.9	698.9	144	526.2	526.2 to 636.9	636.9
8	Sedentary	282	576.2	576.2 to 647.0	647.0	272	595.3	595.3 to 655.8	655.8
	MVPA	282	8.0	8.0 to 15.8	15.8	272	7.0	7.0 to 13.5	13.5
	Overall PA	282	540.1	540.1 to 681.8	681.8	272	488.6	488.6 to 619.0	619.0
<10	Sedentary	198	584.0	584.0 to 650.7	650.7	172	605.3	605.3 to 673.3	673.3
	MVPA	198	8.3	8.3 to 16.3	16.3	172	6.3	6.3 to 12.5	12.5
	Overall PA	198	512.1	512.1 to 667.1	667.1	172	454.2	454.2 to 604.1	604.1

Physical Activity and sedentary tertiles are presented in minutes per day.

4.4 Results

Table 4-2 shows the association of age, birth weight and physical activity variables with BMI in a sex-stratified analysis. All variables, except time spent in vigorous activity for girls, were significantly associated with BMI. The magnitude of the relationship reported on standardized beta coefficients varied between 0.228 and -0.035 SD per unit increase in the explanatory factor in both sexes. Age and birth weight showed the strongest and vigorous activity showed the weakest relationship with BMI. These variables explained between 5.1% and 0.9% of the BMI variance observed for both boys and girls.

The standardised regression coefficient, derived from regression analysis revealed that body weight was significantly associated with age (0.774 in boys and 0.777 in girls), birth weight (0.096 in boys and 0.137 in girls), sedentary behavior (0.120 in boys and 0.150 in girls) and MVPA (0.106 in boys and 0.142 in girls). However body weight shows a negative association with overall physical activity in both sexes (-0.107 in boys and -0.115 in girls) but not with vigorous activity which was only positively associated with body weight in girls (0.097), but not in boys (Table 4.2). All units were estimated using regression analysis and expressed as standardised regression coefficient (as per units of SD changed). The magnitude of the effect was ranging from -0.107 to 0.777 SD per unit change in the explanatory factor. Interestingly, vigorous PA in girls and MVPA in both sexes were positively associated with body weight. Age explained ~59% of the body weight variance while birth weight and physical activity factors explained between 4.0% and 1.1% of the outcome variance.

Table 4.3, shows the association of age, birth weight, ad physical activity factors with waist circumference. Similarly, to the association with body weight, age was strongly related to waist in both sexes, with a size effect of 0.55-0.53 SD per unit change in age (SD) and explains ~31% and 28% of the waist circumference variation in boys and girls respectively. Birth weight and sedentary time were positively associated to waist circumference while overall PA was negatively associated to the outcome in both sexes. These variables explain between 0.4% and 3.8% of the variance of waist circumference in both sexes.

In Table 4.4 subscapular thickness was positively associated with age and sedentary behaviours and negatively associated with MVPA and vigorous PA, but no association was found with birth weight. In Table 4.5, triceps thickness was negatively associated with MVPA, vigorous and overall physical activity was positively associated with age for both sexes but not birth weight which was only significantly associated to triceps in girls. No association was found between sedentary time and triceps thickness. For both skin-folds measurements, the size effects ranged between -0.143 and 0.240 SD, and explained between 5.7% and 0.9% of the skin-fold variance.

To examine whether sedentary behaviour and MVPA are independently associated to the adiposity-related markers, a multiple regression was fitted and age, country and Actigraph

wearing time were added as a covariates (Table 4.6). The analysis revealed that MVPA was significantly associated to all the adiposity markers independent of time spent in sedentary behaviours, age, Actigraph wearing time and country. A similar size effect of MVPA on BMI, waist circumference, subscapular and triceps was found, with a standardized beta coefficient ranging between -0.090 and -0.222 SD for a SD change in MVPA. The association between sedentary behaviours and BMI, weight, subscapular and triceps was abolished after adjusting for time spent in MVPA. However, the association between sedentary behaviour and waist circumference was independent of MVPA, with a standardized coefficient of 0.060 and 0.064 SD per unit change in sedentary time in boys and girls respectively.

Figure 4.1 shows the effect of increasing time spent in sedentary behaviours on body weight, waist circumference, subscapular and tricep in boys and girls. First, an interaction between tertile of sedentary behaviour and sex was fitted to examine if the effect of sedentary time differs by sex. The unadjusted model revealed a significant sedentary time x sex interaction for all the outcomes (BMI p=0.003; body weight p=0.0001; waist circumference p=0.0002; subscapular p=0.003 and tricep p=0.002). However after adjusting for age, Actigraph wearing time and country, these interactions were abolished for all outcomes (BMI p=0.558; body weight p=0.662; waist circumference p=0.953; subscapular p=0.178 and tricep p=0.494). Due to no interactions found after adjusting for confounder factors, a trend analysis was performed for each of the outcome and sex was removed from the interaction and added as a covariate to adjust the model for sex differences. These analyses showed a significant trend to increase BMI (p=0.0007), body weight (p=0.004), waist circumference (p<0.003), subscapular (p<0.005) and tricep (p=0.023) with increasing tertile of sedentary time in country, age, Actigraph wearing time, and sex adjusted model.

The effects of increasing time spent in MVPA on the adiposity-related phenotypes were shown in Figure 4.2. A significant interaction effect was found between tertile of MVPA and sex on BMI (p=0.033), body weight (p=0.048), but not for subscapular (p=0.374), tricep (p=0.295) and waist circumference (p=0.257) in unadjusted model. After accounting for age, Actigraph wearing time and country the MVPA tertile x sex interaction became non- significant for body weight (p=0.551) and BMI (p=*0.*725). For subscapular (p=*0.728*), triceps (p=*0.*613) and waist circumference (p=*0.*705), still remained not significant. Both girls and boys experienced a similar decrease in body weight with increasing time spent in MVPA. On the other hand, BMI was reduced similarly in boys than girls with increasing time spent in MVPA. On those variables where the MVPA x sex interaction was not significant, sex was removed as an interaction factor and fitted in the model as a covariate along with age, Actigraph wearing time and country. This analysis revealed a significant trend to decrease body weight (p<0.0001), BMI (p<0.0001), waist circumference (p<0.0001) sub-scapular (p<0.0001) and triceps skinfold thickness (p<0.0001) with increasing time spent in MVPA.

The effects of overall physical activity on the adiposity-related markers are shown in Figure 4.3. The unadjusted model showed no evidence of an interaction effect between overall PA tertile and sex on the adiposity outcomes (BMI p=0.610; body weight p=0.306; waist circumference p=0.901; subscapular p=0.632 and triceps p=0.576). After adjusting for age, Actigraph wearing time and country, the interaction remained non-significant. Since no significant interaction was found, the sex factor was removed from the interaction and added as a covariate into the model to perform a trend analysis. The analyses showed a significant trend to reduce BMI p<0.0001; body weight p=0.0008; waist circumference p<0.0001; subscapular p=0.0002 and triceps p<0.0001, with increasing time spent in overall physical activity, independent of age, Actigraph wearing time, sex and country.

When a categorical variable was the outcome, logistic regressions were used to estimate the odds of being overweight/obese. First, a sex x PA tertile interaction was fitted into the model to see if the odds of being overweight/obese differ by sex. However, no evidence for an interaction was found (Sedentary, p= 0.702; MVPA, p= 0.610; Overall PA, p= 0.498). The main result showed that increasing doses of sedentary time is related to an increase in the odds of being overweight/obese. Those children on the middle tertile have a risk of being overweight/obese, 18% higher than those in the lower tertile or less inactive. While more inactive children (upper tertile) have a risk of 73% of being overweight/obese compared to those less inactive. This association remained significant after accounting for age, sex, country and overall physical activity; however the risk of being overweight/obese was partially attenuated to 36% after accounting for overall physical activity. These data indicate that the association between sedentary behaviours and obesity could be reduced partially by adopting a physically active lifestyle.

The risk of being overweight/obese was significantly reduced in children that were more active (spent more time in MVPA) compared to the inactive ones (Table 4.7). The risk of being overweight/obese was 28% lower on those individuals in the middle MVPA tertile and 48% on those with higher levels of MVPA (upper tertile) compared to children that were less active (lower tertile). Similarly, overall PA was associated with a lower risk of being overweight/obese. Those children placed in the middle tertile, showed a reduced risk of being overweight/obesity by 36% and those in the upper tertile showed a reduction of 45% compared to the less active children (lower tertile). Further adjustment for potential confounding factors did not alter any of these associations.

Table 4-2 Simple regression coefficients for the association between age, birth weight and physical activity with body mass index in boys and girls of the IDEFICS study.

Outcome (BMI)	Boys				Girls			
Predictors	β (95%CI)	βeta (SE)	p	Adj. r^2	β (95%CI)	βeta (SE)	p	Adj. r^2
Age (years)	0.213 (0.17 to 0.26)	0.184 ± 0.02	<0.0001	3.8%	0.282 (0.23 to 0.33)	0.228 ± 0.02	<0.0001	5.1%
Birth weight (gr) *	0.0003 (0.0002- to 0.0005)	0.097 ± 0.02	<0.0001	0.9%	0.0003 (0.0002 to 0.0005)	0.096 ± 0.02	<0.0001	0.9%
Sedentary time ($min.day^{-1}$) *	0.003 (0.001 to 0.004)	0.058 ± 0.03	0.001	2.1%	0.003 (0.001 to 0.005)	0.088 ± 0.03	<0.0001	2.2%
MVPA ($min.day^{-1}$)*	-0.021 (-0.03 to -0.006)	-0.091 ± 0.02	0.004	1.8%	-0.018 (-0.04 to 0.0006)	-0.060 ± 0.04	0.048	1.1%
Vigorous activity ($min.day^{-1}$)*	-0.057 (-0.10 to -0.01)	-0.076 ± 0.03	0.011	1.6%	-0.028 (-0.08 to 0.02)	-0.035 ± 0.03	0.244	0.9%
Overall Physical Activity ($count.min^{-1}$)*	-0.001 (-0.002 to -0.0004)	-0.097 ± 0.03	0.002	0.9%	-0.002 (-0.002 to -0.0009)	-0.125 ± 0.03	<0.0001	1.5%

Data showed as β coefficient and 95%CI and standardised beta coefficient for each of the outcome. Variables denoted by * were adjusted for age and country.

Table 4-3 Simple regression coefficients for the association between age, birth weight and physical activity with body mass in boys and girls of the IDEFICS study.

Outcome (Body Weight)	Boys				Girls			
Predictors	β (95%CI)	βeta (SE)	p	Adj. r^2	β (95%CI)	βeta (SE)	p	Adj. r^2
Age (years)	2.818 (2.72 to 2.91)	0.774 ± 0.01	<0.0001	59.9%	2.902 (2.80 to 3.00)	0.777 ± 0.01	<0.0001	59.6%
Birth weight (gr) *	0.001 (0.0006 to 0.001)	0.096 ± 0.02	<0.0001	0.9%	0.002 (0.001 to 0.002)	0.137 ± 0.02	<0.0001	1.8%
Sedentary time ($min.day^{-1}$) *	0.013 (0.01 to 0.02)	0.120 ± 0.03	<0.0001	2.6%	0.016 (0.01 to 0.02)	0.150 ± 0.03	<0.0001	4.0%
MVPA ($min.day^{-1}$)*	0.076 (0.03 to 0.12)	0.106 ± 0.03	<0.0001	1.4%	0.131 (0.07 to 0.19)	0.142 ± 0.03	<0.0001	2.6%
Vigorous activity ($min.day^{-1}$)*	0.042 (0.09 to 0.18)	0.018 ± 0.04	0.557	0.3%	0.23 (0.07 to 0.38)	0.097 ± 0.03	0.003	1.5%
Overall Physical Activity ($count.min^{-1}$)*	-0.004 (-0.006 to -0.001)	-0.107 ± 0.03	0.001	1.1%	-0.005 (-0.008 to -0.002)	-0.115 ± 0.02	<0.0001	1.2%

Data showed as β coefficient and 95%CI and standardised beta coefficient for each of the outcome. Variables denoted by * were adjusted for age and country.

Table 4-4 Simple regression coefficients for the association between age, birth weight and physical activity with Waist Circumference in boys and girls of the IDEFICS study.

Outcome (Waist Circumference)	**Boys**				**Girls**			
Predictors	β (95%CI)	βeta (SE)	p	Adj. r^2	β (95%CI)	βeta (SE)	p	Adj. r^2
Age (years)	1.934(1.81 to 2.05)	0.559 ± 0.01	<0.0001	31.3%	1.931(1.80 to 2.06)	0.535 ± 0.01	<0.0001	28.7%
Birth weight (gr) *	0.0007 (0.0003 to 0.001)	0.068 ± 0.02	0.001	0.4%	0.0008(0.0004 to 0.001)	0.077 ± 0.02	<0.0001	0.5%
Sedentary time ($min.day^{-1}$) *	0.013 (0.01 to 0.02)	0.149 ± 0.03	<0.0001	2.6%	0.016 (0.01 to 0.02)	0.177 ± 0.03	<0.0001	3.8%
MVPA ($min.day^{-1}$)*	0.007 (-0.03 to 0.05)	0.009 ± 0.03	0.742	0.01%	-0.015 (-0.07 to 0.04)	-0.017 ± 0.03	0.591	0.%
Vigorous activity ($min.day^{-1}$)*	-0.081 (-0.21 to 0.05)	-0.037 ± 0.031	0.228	0.3%	-0.034 (-0.18 to 0.11)	-0.014 ± 0.03	0.641	0.2%
Overall Physical Activity ($count.min^{-1}$)*	-0.005 (-0.007 to -0.003)	-0.140 ± 0.03	<0.0001	1.9%	-0.007 (-0.009 to -0.004)	-0.168 ± 0.032	<0.0001	2.7%

Data showed as β coefficient and 95%CI and standardised beta coefficient for each of the outcome. Variables denoted by * were adjusted for age and country.

Table 4-5 Simple regression coefficients for the association between age, birth weight and physical activity with Sub-Scapular in boys and girls of the IDEFICS study.

Outcome (Sub-Scapular)	**Boys**				**Girls**			
Predictors	β (95%CI)	βeta (SE)	p	Adj. r^2	β (95%CI)	βeta (SE)	p	Adj. r^2
Age (years)	0.238 (0.18 to 0.29)	0.166 ± 0.02	<0.0001	2.7%	0.448 (0.37 to 0.52)	0.240 ± 0.02	<0.0001	5.7%
Birth weight (gr) *	-7.650 (-0.0002 to 0.0001)	-0.001 ± 0.02	0.937	0.001%	-7.260 (-0.0002 to 0.0002)	-0.0001 ± 0.02	0.996	0.001%
Sedentary time ($min.day^{-1}$) *	0.003 (0.001 to 0 .005)	0.067 ± 0.03	0.003	1.0%	0.014 (0.004 to 0.009)	0.119 ± 0.03	<0.0001	2.5%
MVPA ($min.day^{-1}$)*	-0.036 (-0.05 to -0.02)	-0.125 ± 0.03	<0.0001	1.7%	-0.065 (-0.09 to -0.04)	-0.137 ± 0.03	<0.0001	2.2%
Vigorous activity ($min.day^{-1}$)*	-0.104 (-0.16 to -0.04)	-0.106 ± 0.03	0.0006	1.3%	-0.140 (-0.22 to -0.06)	-0.113 ± 0.03	<0.0001	1.6%
Overall Physical Activity ($count.min^{-1}$)*	-0.002 (-0.003 to -0.001)	-0.143 ± 0.03	<0.0001	1.9%	-0.004 (-0.005 to-0.002)	-0.172 ± 0.03	<0.0001	2.8%

Data showed as β coefficient and 95%CI and standardised beta coefficient for each of the outcome. Variables denoted by * were adjusted for age and country.

Table 4-6 Simple Regression coefficients for the association between age, birth weight and physical activity with Triceps in boys and girls of the IDEFICS study.

Outcome (Triceps)	**Boys**				**Girls**			
Predictors	β (95%CI)	βeta (SE)	p	Adj. r^2	β (95%CI)	βeta (SE)	p	Adj. r^2
Age (years)	0.268 (0.19 to 0.34)	0.141 ± 0.02	<0.0001	1.9%	0.489 (0.39 to 0.58)	0.223 ± 0.02	<0.0001	4.9%
Birth weight (gr) *	0.0002 (-0.00004 to 0.0004)	0.035 ± 0.02	0.102	0.15%	0 .0004 (0.0001 to 0.0007)	0.061 ± 0.005	0.006	0.3%
Sedentary time ($min.day^{-1}$) *	0.002 (-0.0004 to 0.005)	0.026 ± 0.03	0.090	0.5%	.003 (-0.0003 to 0.006)	0.027 ± 0.03	0.079	1%
MVPA ($min.day^{-1}$)*	-0.054 (-0.08 to -0.03)	-0.143 ± 0.03	<0.0001	2.3%	-0.073 (-0.107 to -0.04)	-0.131 ± 0.03	<0.0001	2.5%
Vigorous activity ($min.day^{-1}$)*	-0.14 (-0.22 to -0.06)	-0.109 ± 0.03	<0.0001	1.1%	-0.141 (-0.23 to -0.05)	-0.098 ± 0.03	0.002	0.9%
Overall Physical Activity ($count.min^{-1}$)*	-0.003 (-0.004 to -0.001)	-0.126 ± 0.03	<0.0001	1.6%	-0.003 (-0.005 to -0.002)	-0.132 ± 0.03	<0.0001	1.7%

Data showed as β coefficient and 95%CI and standardised beta coefficient for each of the outcome. Variables denoted by * were adjusted for age and country.

Table 4-7 Multiple regression coefficients for the association of sedentary behaviour and MVPA with adiposity-related markers.

Outcome	Predictors/ Outcome	**Boys**				**Girls**			
		β (95%CI)	βeta (SE)	p	Adj. r^2	β (95%CI)	βeta (SE)	p	Adj. r^2
BMI	Sedentary time (min.day^{-1}) *	0.001 (-0.0004 to 0.002)	0.046 ± 0.02	0.158	0.2%	0.001 (-0.0005 to 0.003)	0.047 ± 0.03	0.160	0.02%
	MVPA (min.day^{-1})*	-0.031 (-0.045 to -0.017)	-0.138 ± 0.03	0.0001	1.9%	-0.043 (-0.063 to -0.022)	-0.139 ± 0.03	0.0001	1.9%
Weight	Sedentary time (min.day^{-1}) *	0.001 (-0.001 to 0.005)	0.019 ± 0.02	0.380	0.03%	0.001 (-0.002 to 0.005)	0.015 ± 0.03	0.488	0.02%
	MVPA (min.day^{-1})*	-0.063 (-0.092 to -0.034)	-0.090 ± 0.03	0.0001	0.8%	-0.088 (-0.128 to -0.047)	-0.095 ± 0.02	0.0001	0.9%
Waist	Sedentary time (min.day^{-1}) *	0.004 (0.0003 to 0.008)	0.060 ±0.01	0.034	0.3%	0.005 (0.0004 ± 0.009)	0.064 ± 0.03	0.030	0.4%
	MVPA (min.day^{-1})*	-0.082 (-0.117 to -0.047)	-0.125 ±0.02	0.0001	1.5%	-0.152 (-0.202 to -0.101)	-0.174 ±0.03	0.0001	3.0%
Subscapular	Sedentary time (min.day^{-1}) *	0.001 (-0.001 to 0.003)	0.030 ± 0.04	0.370	0.09%	0.002 (-0.0003 to 0.005)	0.057 ± 0.04	0.091	0.3%
	MVPA (min.day^{-1})*	-0.052 (-0.071 to -0.033)	-0.177 ± 0.03	0.0001	3.1%	-0.104 (-0.135 to -0.073)	-0.222 ± 0.03	0.0001	4.9%
Tricep	Sedentary time (min.day^{-1}) *	-0.006 (-0.003 to 0.002)	-0.015 ± 0.06	0.645	0.02%	-0.001 (-0.004 to 0.002)	-0.025 ± 0.04	0.453	0.06%
	MVPA (min.day^{-1})*	-0.075 (-0.099 to -0.051)	-0.198 ± 0.03	0.0001	3.9%	-0.117 (-0.153 to -0.080)	-0.215 ± 0.02	0.0001	4.6%

Data showed as β coefficient and 95%CI and standardised beta coefficient for each of the outcome. Age, country and Actigraph wearing time were added as covariates.

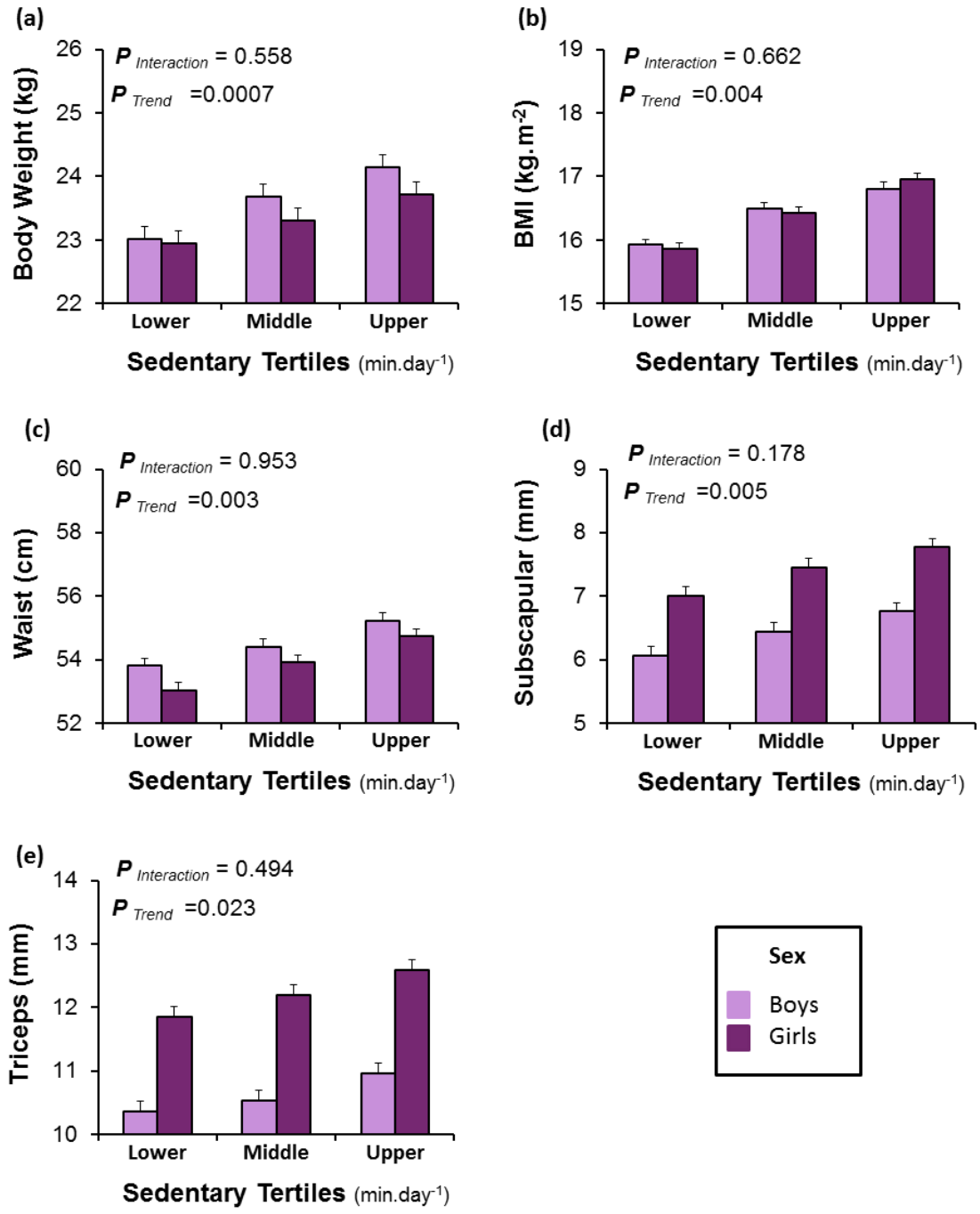


Figure 4.1 Effects of Time spent on sedentary behaviours on BMI, Body Mass, Waist circumference, Subscapular and Triceps in European Children.

Data presented as adjusted mean ± SEM. Full adjusted p-value for interaction between physical activity and sex were given for each of the outcomes. When the interaction was not significant the p-trend was given for each of the outcome and sex was added as a covariate in the model along with age, Actigraph wearing time and country. Tertiles were age and sex specific as described in Table 4-1.

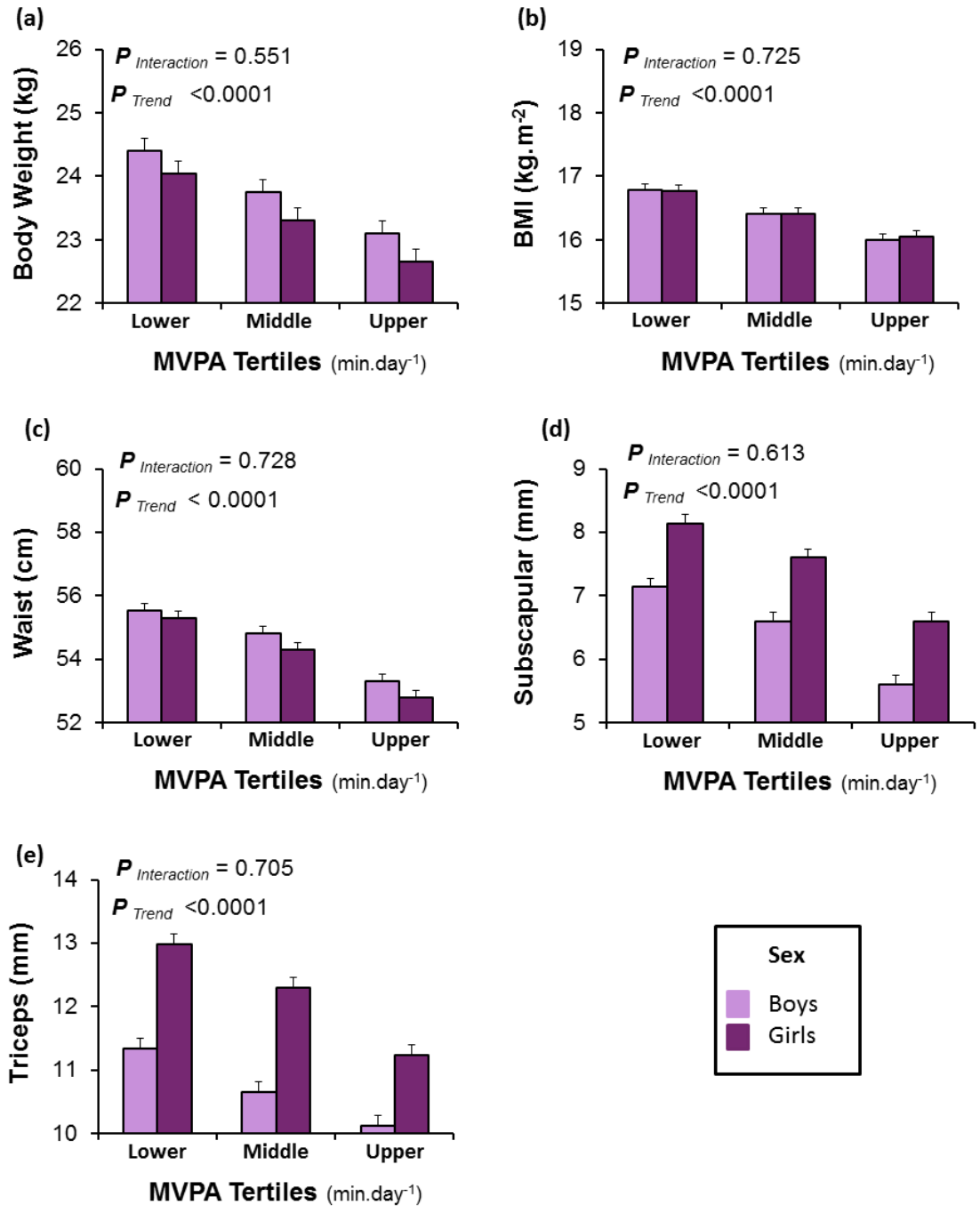


Figure 4.2 Effects of time spent on MVPA on BMI, body mass, waist circumference, subscapular and triceps in European children.

Data presented as adjusted mean ± SEM. Full adjusted p-value for interaction between physical activity and sex were given for each of the outcomes. When the interaction was not significant the p-trend was given for each of the outcome and sex was added as a covariate in the model along with age, Actigraph wearing time and country. Tertiles were age and sex specific as described in Table 4-2.

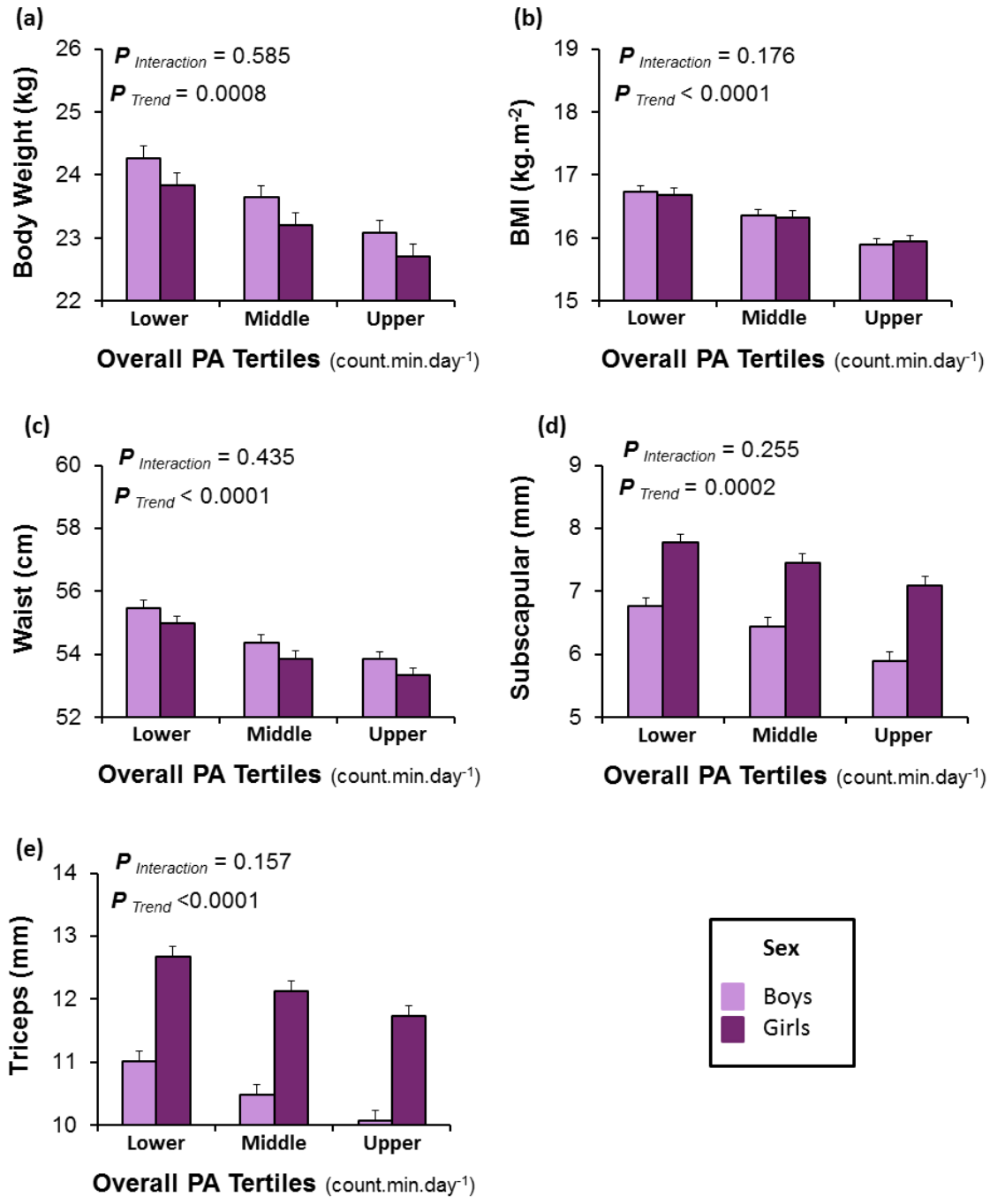


Figure 4.3 Effects of time spent on overall physical Activity on BMI, body mass, waist circumference, subscapular and triceps in European children.

Data presented as adjusted mean ± SEM. Full adjusted p-value for interaction between physical activity and sex were given for each of the outcomes. When the interaction was not significant the p-trend was given for each of the outcome and sex was added as a covariate in the model along with age, Actigraph wearing time and country. Tertiles were age and sex specific as described in Table 4-3.

Table 4-8 Odd ratios of being overweight/obese by tertiles of physical activity and sedentary behaviour in children.

	Model 0 OR (95%CI)	P-value	Model 1 OR (95%CI)	P-value	Model 2 OR (95%CI)	P-value
Tertiles of MVPA						
Middle	0.72 (0.68 to 0.93)	<0.0001	0.78 (0.53 to 0.89)	<0.0001	0.78 (0.60 to 0.89)	0.001
Higher	0.52 (0.49 to 0.61)	<0.0001	0.44 (0.33 to 0.67)	<0.0001	0.51 (0.43 to 0.67)	<0.0001
Tertiles of Sedentary Behaviours						
Middle	1.18 (1.08 to 1.39)	<0.0001	1.23 (1.04 to 1.44)	<0.0001	1.19 (1.02 to 1.47)	<0.0001
Higher	1.73 (1.48 to 1.83)	<0.0001	1.50 (1.21 to 1.68)	<0.0001	1.36 (1.12 to 1.55)	<0.0001
Tertiles of Overall PA						
Middle	0.64 (0.40 to 0.71)	<0.0001	0.77 (0.26 to 0.83)	<0.0001	0.74 (0.64 to 0.98)	<0.0001
Higher	0.55 (0.49 to 0.63)	<0.0001	0.44 (0.50 to 0.78)	<0.0001	0.59 (0.41 to 0.83)	<0.0001

Data presented as OR (95%CI). Age and sex specific tertiles of physical activity/inactivity were derived from the continuous variable and fitted into the model as ordinal variables (Low=0, Middle=1, Upper=2). Overweight and obese were combined and normal weight was used as a reference group.
Model 0, unadjusted.
Model 1, adjusted for age, sex and country.
Model 2, adjusted for age, sex, country, Actigraph wearing time, and PA variables (when sedentary time was the predictor overall PA was added as a covariate, and when MVPA or overall PA was the predictor sedentary time was added as a covariate).

4.5 Discussion

The main finding of this study was that MVPA was significantly associated with all the adiposity markers independent of time spent in sedentary behaviour, age, Actigraph wearing time and country. However, the adverse association of sedentary time on adiposity related markers was abolished when MVPA was included in the model; suggesting that high levels of activity in children may attenuate the health related risk of inactivity. This finding has important public health implications as time spent in sedentary behaviours has increased substantially in children over the last few decades. However, this suggests that independent of how much time children sitting or in sedentary-related behaviours, if they meet the recommendation and increase their physical activity the detrimental health effect of inactivity is partially abolished.

Sedentary behaviour is an important area of study in health research. It is defined as any waking behaviour associated with an energy expenditure of ≤1.5 METs and a sitting or reclining posture, and is considered separate and distinct from a lack of moderate- to vigorous-intensity physical activity (i.e., not meeting specified physical activity guidelines) (Network 2012). Research on school-age children (aged 5–10 years) indicates that on average they spend 8.6 h per day, or 62% of their waking hours engaging in sedentary behaviour (Colley et al. 2011). These sedentary activities, especially those that are screen-based, are associated with increased risk for obesity, decreased fitness, self-esteem, pro-social behaviour, and academic achievement (Tremblay et al. 2011). However, limited data is available about doses of sedentary behaviour and its effect on health-related parameter.

Accumulating evidence using self-reported measurements indicate sedentary lifestyles are also occurring during the early years. For example, several sources report that children in their early years spend 73%–84% of their waking hours being sedentary (Vale et al. 2010). Consequently, there is an increased interest in the health implications of excessive sedentary behaviour during this critical period of growth and development. Evidence suggest that compared with school-aged children, screen time may be associated with additional negative health outcomes during the early years (Lillard and Peterson 2011). Furthermore, sedentary behaviour habits formed during the early years may track over time (Janz et al. 2005). As a result, there may be several immediate and long-term health benefits by encouraging appropriate sedentary behaviour habits in this age group. However, the results of these previous studies do not provide specific information neither on the dose of sedentary behaviour necessary for good health, nor do they provide definitive information as to how this relationship differs between boys and girls. All studies reported on the relationship between television viewing and a health indicator (i.e., no other types of sedentary behaviour were explored). It is important to highlight that television viewing is only a crude measure of sedentary behaviour and it is likely that caregivers underestimate this time, meaning that our results may be in fact underestimating television viewing and its overall impact on poor health. Therefore, IDEFICS study provides a more accurate representation of how sedentary behaviours objectively measured relate to obesity-related

markers using large cohorts of European children so that groups can be stratified by volume of sedentary behaviour, sex, and age group.

IDEFICS provide an objective picture of sedentary behavior and its dose-effect relationship on European children. Although it is evident that physical activity levels and high levels of sedentary behavior may be related to obesity and health parameters in children, there is limited objective data on PA and its relationship with children health. In this study, the author reports that a similar size effect of MVPA on BMI, waist circumference, subscapular and tricep skinfolds were observed, supporting an essential role of physical activity in preventing obesity effects in young children. The findings of this study were in agreement with previous work in this field which supports strong dose-response association between PA and obesity (Ness et al. 2007; Jimenez-Pavon et al. 2009). In addition, the findings of this study support the notion that increased sedentary behaviour is not necessarily equivalent to a lack of physical activity, suggesting that children who participate in some kind of physical activity may be protected from becoming overweight or obese. The results of this study are consistent with results of a meta-analysis which concluded that sedentary behaviour was not associated with physical activity or BMI (Marshall et al. 2004). It was also observed that the relative risk or association of obesity phenotypes did not differ between the two sexes when MVPA was added into the model.

The results of this study demonstrate that levels of MVPA are associated with obesity traits thus confirming more overweight or obese children are involved in less amount of PA. The current literature suggests that decreased levels of PA in children is associated with levels of obesity, hypertension, cardiovascular disease which include metabolic syndrome (Mountjoy et al. 2011). Studies have estimated that metabolic syndrome is observed in 3-14% of all youth and it is increasing along with obesity (de Ferranti et al. 2004; Weiss et al. 2004; Ford et al. 2005; Jolliffe and Janssen 2007). Moreover, a study assessing the associations of physical activity with cardiovascular disease in children, suggests that activity levels should be higher than the current international guidelines which state that at least 1 hour per day of physical activity of at least moderate intensity is needed to prevent clustering of cardiovascular disease risk factors (Andersen et al. 2006). Additionally, physical activity aids not only in the physical health of children but also helps improve their mental health and self-esteem. In a recent study which aimed to examine the relationship between physical activity and wellbeing in children, it was observed that children who meet the recommended guidelines of MVPA (60 minutes MVPA per day) were more likely to have better wellbeing (Breslin et al. 2011). Although the evidence of the health benefits of PA are becoming stronger, there is an alarming trend of children's PA levels declining as they move through adolescence (Nader et al. 2008; Corder et al. 2010). This should be considered when designing interventions and PA promotion strategies in specific target age groups.

A particular strength of the study is the detailed phenotype data of the study population; particularly with respect to lifestyle measures. Physical activity and sedentary time were

objectively measured, providing greater validity in these measures than what would be obtained from self-report questionnaires (Shephard 2003). Due to the fact that this study is cross-sectional in nature, it is not possible to draw firm conclusions about the causality of associations observed. A randomized controlled trial is needed to address this definitively. However, this study design, with relatively precise measures of a number of relevant exposure variables, allowed us to control for a number of potential confounding variables in our analyses. Although potential residual confounding effects cannot be excluded, the robustness of our findings to these adjustments supports our conclusions.

Furthermore, the use of accelerometry devices for the collection of activity data can be also considered a strength of this study, as most self-reporting instruments appear to overestimate both the duration and intensity of PA (Adamo et al. 2009). However, self-report studies support that the PA levels have not declined during the past decade (Li et al. 2009). Interestingly, evidence show that the prevalence of sufficiently active youth measured by accelerometry, ranged between 1% and 100%, depending on the intensity thresholds used, suggest that sport participation is likely to contribute to higher levels of PA (Ekelund et al. 2011). As a result, when assessing the number of children meeting the activity guidelines, it is important to validate the assessment method and the intensity thresholds used when accelerometry is the objective measure of PA. Limitations of the accelerometry use in young people can be considered the challenging interpretation of the data collected, as well as the fact that there is lack of information about the types of PA, the setting and the contexts in which PA takes place as well as the amount of PA devoted to specific domains (Mountjoy et al. 2011). In addition, when using accelerometry devices it is possible to observe the *"Hawthorne effect",* a general scientific fact that the process of observation alters the phenomenon being observed (Corder et al. 2008) in that it may alter one's behaviour. In a study of validating methods and protocols, accelerometer counts were found to be 3% higher during the first day of measurement than subsequent days in 11-yr-old children (Mattocks et al. 2008), suggesting an initial awareness of observation. Because the problem is not apparent on subsequent days, one solution may be to discount the first day entirely or to scale it. Furthermore, it is vital to highlight the importance of the representativeness period when monitoring physical activity. In this study children had to wear a device during waking hours for three consecutive full days. Ideally, two of the days were weekdays and the other one weekend day. However there is evidence that between 4 and 9 full days of monitoring, including 2 weekend days is required for a reliable estimate in youth (Trost et al. 2005). Seven days of continuous monitoring seems efficient enough, but because protocol adherence tends to decrease with days of wear, it may be more feasible to decide on four full days with at least 1 weekend day, as it is often done in large studies (Riddoch et al. 2004). More studies are necessary in order to clarify whether initial estimates of 7 day PA monitoring captures a sufficiently high level of inter individual variation and are reasonable for surveillance and intervention studies (Matthews et al. 2012). In addition, in a large study of 11-yr-old children that examined the number of days of monitoring required to achieve reliability coefficients of 0.7, 0.8, and 0.9, the trade-off between feasibility and accuracy was demonstrated (Mattocks et

al. 2008). Three days of monitoring were required to achieve a coefficient of 0.7, five days were required for a coefficient of 0.8 and 11 days were needed to achieve a reliability coefficient of 0.9. There also appears to be an age effect, with younger children having less day-to-day variability than older children with regards to MVPA, as it has been shown that 4–5 days of monitoring achieves a reliability coefficient of 0.80 in young children, but between 8 and 9 days may be required for adolescents (Trost et al. 2000) Therefore, due to differences between weekdays and weekend days along with the balance between feasibility and validity, it is recommended that as a minimum, studies in both children and adolescents should aim for at least 4 full days of monitoring including one weekend day (Corder et al. 2008).

Furthermore, the existence of multiple sets of intensity-related cut points for children and adolescents has significantly delayed research efforts to accurately quantify and understand physical activity behaviour of youth. To date, at least five sets of youth-specific Actigraph cut points have been independently developed and published in the peer-reviewed scientific literature (Puyau et al. 2002; Treuth et al. 2004; Freedson et al. 2005; Mattocks et al. 2007; Evenson et al. 2008). Importantly, the method used to derive these cut points varied considerably from study to study. For example, for some cut points a large age range was used, whereas others were derived from a narrow age range or single age group. Some were derived from a single-sex group, whereas others were derived from both sexes. Some were based on direct measures of EE, whereas others were based on direct observation. In addition, the methods also vary in the activities measured and whether they were based on fixed (i.e. treadmill) or free-play and lifestyle activities. Additionally, some equations provided age-specific count cut points, whereas the others derived a single cut point for children and adolescents of different ages. Another limitation is the use of low MVPA cut points for children, which was found to be associated with an increased likelihood of misclassifying light-intensity activities as moderate, thus leading to potential overestimations of time spent in MVPA (Trost et al. 2010). It is clear that the existence of different cut points predicting the activity intensity in youth, and the lack of consensus on cut point selection, has challenged the researchers interested in the understanding of PA behaviour of youth.

In conclusion, the present study reveals that physical activity influences adiposity in children of European descent. These associations persist after adjustment for a comprehensive range of potential confounding factors. However, physical activity, and more specifically MVPA, influenced obesity related variables in a similar manner in both sexes. However, the cross-sectional nature of these data does not allow firm conclusions to be drawn about causality, but the findings emphasize the fact that environmental and lifestyle factors on obesity risk have a significant role in the development or prevention of childhood obesity, and suggest that further studies into the mechanisms underpinning this effect are needed. This has potential value for both the design and implementation of lifestyle strategies to reduce obesity risk in different age groups, and advancing the basic understanding of the mechanisms underpinning childhood

obesity. Finally, further research is necessary to develop effective strategies for motivating young children to become more active and discourage the sedentary behaviour.

5 Association between *FTO* gene and obesity-related phenotypes: its effect on physical activity

5.1 Introduction

The previous chapter showed that lifestyle factors such as time spent in different intensities of physical activity are related to an increase in obesity-related phenotypes. This is in agreement with previous studies which show that environment and lifestyle factors affect the increased prevalence of obesity in a significant way (Hill et al. 2003). In addition, although environment seems to be a strong contributor to obesity, it does not seem to explain all the variance of obesity prevalence which highlights the multi-factorial origin of this health problem. This supports the idea that there could be more factors influencing the prevalence of obesity and genetic predisposition might have a key role in this. In addition, twin studies indicate a heritability of fat mass between 40-70% (Poulsen and Vaag 2003), suggesting that genetic effect is an important contributor to obesity. Moreover, evidence show that adopted children have BMIs that correlate better with those of the biological parents than the fat mass of the adoptive parents (Maes et al. 1997); again supporting a genetic role in the predisposition to obesity. One of the largest recent meta-analysis of genetic studies on BMI in pre-adolescence, young adulthood and late adulthood have shown evidence that heritability of BMI remains high over the age categories, with estimates of 0.75 (95 % CI 0.70–0.80) in pre-adolescence, 0.80 (95 % CI 0.76–0.81) in young adulthood, and somewhat lower in late adulthood at 0.61 (95 % CI 0.54–0.64) (Nan et al. 2012). The outcome of this meta-analysis also revealed that common environmental factors are less influential on individual difference in BMI in late adulthood, whereas unique environmental influences increase steadily from pre-adolescence to late adulthood. This pattern is not surprising, as we expect their interests and lifestyles to diverge as twins separate from their shared household (Nelson et al. 2006).

FTO is a very large gene whose nine exons span more than 400 kb on chromosome 16. The SNPs identified by these three studies are located in the first intron of the gene, a region where the sequence is strongly conserved across species. They represent a cluster of at least 40 SNPs that are highly correlated (linkage disequilibrium r2 > 0.80 in CEU of the HapMap) in Caucasian populations(Loos and Bouchard 2008). It is therefore expected that the result would have been similar if we had genotyped other SNPs of the FTO gene (rs8050136, rs7193144, rs1421085, rs1121980 or rs9939609), due to the strong linkage disequilibrium (LD) as they are perfect proxies for each other. The *FTO* (fat mass and obesity-associated gene) was the first to be implicated in a human phenotype in 2007 for Type 2 diabetes. This study revealed that *FTO*

was strongly associated with the fat mass in the general European population (Frayling 2007). In addition, genome association scans have demonstrated that variants in the *FTO* gene are strongly associated with several obesity phenotypes including increased BMI, waist circumference and body weight, suggesting that *FTO* may be associated with obesity (Scuteri et al. 2007). Moreover, a quantitative trait study conducted in European descent population demonstrated that approximately 16% of individuals homozygous for the risk allele, weighed on average 3 kilograms more than control (Dina et al. 2007).

Several studies have been conducted using young children in order to identify the age at which *FTO* (rs9939609) starts to have an apparent association with BMI (Haworth et al. 2008; Fang et al. 2010). Researchers have suggested that the *FTO* variant was not associated with fetal growth but the changes in BMI were evident by the age of 7, which persisted into the pre-pubertal period (Frayling et al. 2007). Evidence from studies in children also suggest that unlike high risk alleles, the two copies of the lower risk allele have a protective effect against overeating by promoting responsiveness to internal signals of satiety (Wardle et al. 2009). This suggests that genetic predisposition to obesity could be affected by environmental factors such as dietary intake. However, further investigation is needed in order to better understand the role of the gene-environment relationship.

Earlier studies have reported that FTO is highly expressed in the hypothalamus, a region involved in appetite regulation. FTO has been shown to be associated with increased energy intake, especially fat intake (Cecil et al. 2008; Timpson et al. 2008; Wardle et al. 2009) and impaired satiety responsiveness (Wardle et al. 2008) in children. Studies on adults have also shown that risk allele carriers consume more energy (Speakman et al. 2008), whereas the genotypes do not seem to influence energy expenditure (Berentzen et al. 2008; Franks et al. 2008). These results suggest that FTO could alter obesity mainly by having a role in appetite regulation. However, in our study dietary patterns and their relation with FTO were not examined and this could be taken into consideration for future work on the gene-environment investigation of the IDEFICS project. In addition, dietary data can be challenging to interpret as studies have shown that obese individuals generally report to consume less or the same amount of energy as normal weight individuals (Hill and Davies 2001), and studies using the doubly labeled water technique have provided evidence of systematic misreporting of dietary intake among overweight or obese individuals (Lissner et al. 1998; Lissner 2002). Thus, dietary data collection and interpretation may even be more challenging when studying young children.

Despite recent progress, the mechanisms by which SNPs in *FTO* influence human body mass remain elusive. Multiple processes could reasonably contribute to the risk of obesity, including neurological circuits governing appetite and whole-body energy expenditure, as well as peripheral pathways involved in energy expenditure. Loss of *FTO* function appears to reduce fat mass in mice, at least in part, through increased energy expenditure but not decreased energy

intake (Church et al. 2009; Church et al. 2010). However, the studies of intermediate phenotypes in humans showed that *FTO* SNPs are associated with appetite and food intake but not energy expenditure (Speakman et al. 2008; Haupt et al. 2009). Interestingly, data from rodent studies suggest that *FTO* might affect neuropeptide Y expression in hypothalamus, which in turn is known to impact feeding behaviour (Larder et al. 2010). An investigation of the association between *FTO* SNPs expression and neuropeptide levels in the human hypothalamus might therefore provide a potential mechanism for the modulatory effect of *FTO* SNPs on appetite. At present, the strongest associations between *FTO* SNPs and obesity belong to intronic SNP. It is possible that by fine-mapping the causal variant(s) could shed light on the biological mechanism impacting obesity. However, fine-mapping the association signal might be difficult because the obesity-associated SNPs lie within a 47 kb LD block in which the effects of causal variant(s) could be indistinguishable from others. Under these circumstances, it might prove more important to understand the biological effect of the risk haplotype (rather than the causal variants themselves) on genes and pathways.

Physical activity is another environmental factor that may affect the genetic predisposition to obesity related traits. The effect of *FTO* on obesity phenotypes has been studied and was found to be altered by levels of physical activity in adolescents (Scott et al. 2010). Individuals meeting the daily physical activity recommendations were able to overcome the *FTO* effect on obesity related traits (Ruiz et al. 2010). Indeed, the effect of the *FTO* rs9939609 polymorphism on body fat parameters was much lower in adolescents who met the daily physical activity recommendations (60 min/day of moderate MVPA) compared with those who did not: (0.17 vs 0.65 units per risk allele in BMI, respectively; 0.40% vs 1.70% per risk allele in body fat percentage, respectively; and 0.60 vs 1.15 cm per risk allele in waist circumference, respectively. This again highlights that physical activity is an important factor for modifying the effect of *FTO* genotype. In contrast, other studies do not confirm neither an *FTO*-physical activity association nor that physical activity can modulate the *FTO* obesity effects (Hakanen et al. 2009; Liu et al. 2010; Kilpelainen et al. 2011). However, in most studies physical activity was measured via self-reported questionnaires and not accelerometry, which is considered to be a more accurate method. This highlights the need to undertake more gene-environment studies with efficient measures of physical activity in order to achieve accurate outcomes and valid conclusions. The IDEFICS study allows for the investigation of *FTO*-Physical activity relationship on a large number of subjects (young children) using the most efficient measure of PA (accelerometry). In addition to the physical activity patterns being accepted as environmental factors, evidence show that activity behaviour and individual differences may have a genetic background. According to twin studies, up till age 13–14, genes are of no importance in explaining individual differences in exercise participation, whereas a large familial resemblance is found through common environmental effects. In late adolescence (from approximately age 17–18 years old), genetic factors start to appear, the role of common environment decreases and genetic factors peak in their contribution to exercise behaviour around age of 19–20 years old, to decrease again from young adulthood onward to reach a stable value of about 50% in middle-

aged subjects (Stubbe 2009). It has been suggested that genetic influences on exercise ability, which are very strong both for strength and endurance phenotypes (Arden and Spector 1997; Thomis et al. 1997), may explain part of the heritability of exercise behaviour (Stubbe et al. 2006). The fact that PA behaviour may reflect differences in genetic make-up, encourage researchers to change the prospective from looking at "population-based" intervention strategies to "personalized" intervention strategies. Therefore one may hypothesize that isolating the genes responsible for "exercise behaviour" will help find the genes related to obesity related traits and physical health.

Elucidating the relationship between genetic and environmental factors will help us better understand the pathogenesis of obesity and also aid in the design of personalized treatments for the affected population. By improving the current knowledge of the gene-environment relationship will help elucidate how lifestyle factors are able to modulate genetic contribution to risk of complex diseases or conditions such as obesity. This will also allow for better and more efficient design of intervention studies and community based programs, in order to promote prevention of childhood and adult obesity and other related diseases.

The aims of the present chapter are:

(a) To investigate the association between the *FTO* gene and obesity-related phenotypes (weight, BMI, waist circumference and body fat measures) in European Children.

(b) To investigate whether levels of physical activity (Sedentary time, MVPA, Vigorous Time and Overall physical activity) modulate any associations with obesity-related phenotypes in this cohort.

5.2 Methods

A cohort of 16223 children aged 2–9 years was recruited into a population-based baseline survey from eight European countries. Sampling recruitment was related to the geographical location of the participating centers and all schools and kindergartens of each area where asked to be included and participate in the IDEFICS study. After meeting the inclusion criteria for genotyping (weight and height data available), a certain number of subjects were chosen for genotyping from each country. This sample selection was random and aimed for similar numbers of boys and girls from each country. The total sample selected for the genotyping studies was 4407 children, and was extracted from the whole IDEFICS population participating in the baseline survey. This sub-sample of children was genotyped in order to assess the possible effect of *FTO* rs9939609 on each individual's BMI variation. The IDEFICS cohort provided at least 90% power to detect a 20% difference between AA and TT homozygotes at a significance level of $\alpha = 0.05$, assuming an additive mode of inheritance. Volunteers were recruited and screened as detailed in section 2.4. The assessment of anthropometric characteristics, body composition, physical activity, socio-

economic status, and genetic material collection has been described in detail in Chapter 2 (section 2.2).

5.2.1 DNA Preparation and Genotyping Methods

Approximately 2 ml of saliva was collected from children who were able to provide a sputum sample (Oragene DNA Self Collection Kit, tube format OG-300; DNA Genotek Inc., Canada), and sponges (Oragene DNA Self Collection Kit, disc format OG-250 and CS-1 sponge accessory; DNA Genotek Inc., Canada) were used to soak up as much saliva as possible from the inside of the mouths of younger children unable to spit. Upon arrival at the central laboratory at UGLW, samples were logged using a barcode reader system and stored at 4°C pending DNA extraction.

5.2.2 Genomic DNA: Extraction, Storage, Concentration

From each 2ml saliva sample, 500 µl were transferred into a 1.5 ml microcentrifuge tube and the remaining 1.5 ml of the sample was re-sealed in the original collection vessel and frozen at -20°C. Samples were extracted in batches of 24 or 48 using the protocol for manual purification of DNA from saliva, advocated by the manufacturer. Aliquots of 167 µl were quantified using the Nanodrop Technologies Nanodrop® ND-8000 Spectrophotometer (Wilmington, DE, USA) measuring 8 samples at a time, using a multichannel pipette to transfer 1.5 µl of the undiluted sample to the sample platform.

DNA concentrations were estimated from absorbance readings at 260nm (A_{260}) using a 1 O.D. unit = 50 µg/ml conversion factor. A_{260}/A_{280} ratios were also measured. DNA was diluted to a working concentration of 10 ng/µl in TE buffer in 2 ml 96 well plates, and subsequent aliquots were dispensed into 1 ml deep well plates (Starlabs UK Ltd, Buckinghamshire, UK) before storing the original samples in 2 ml plates at -20°C. A re-reading was made to verify the target concentration (10 ng.µl^{-1}) for each sample. The working samples were held at 4°C for several weeks during the genotyping analysis.

5.2.3 Genotyping

The DNA extraction and standardization was conducted by the author of the thesis, however the *FTO* genotyping was performed in Italy, at the ISA-CNR lab which is one of the IDEFIC's genetics team. Genotyping of *FTO* SNPs rs9939609 was performed using TaqMan SNP Genotyping Assays (Applied Biosystems, Warrington, UK), fluorescence was measured using endpoint reads and allele determination using ABI 7900 sequence Detection System, following manufacturer's instructions (Applied Biosystems, Warrington, UK). Allelic discrimination reaction mix was performed using 2.50µl TaqMan® Universal PCR Master Mix and 0.25µl. SNP Genotyping Assay Mix. The genotyping success rate of the *FTO* SNP was 96.2%. The reasons behind the entire gene not being sequenced were related to IDEFICS policies and funding related decisions that were set prior to data collection.

5.3 Statistical analysis

A goodness-of-fit chi-square test (χ^2) (with 1 degree of freedom) was performed to confirm whether the observed genotype counts were in Hardy-Weinberg equilibrium. A General Linear Model (GLM) statistical framework was used to test quantitative variables for differences between genotype groups. The main outcomes used in our analyses were obesity-related phenotypes (weight, BMI, waist circumference, body fat). An additive genetic model was assumed, genotypes were coded (0, 1 and 2) and regression analysis was performed to estimate per allele effect sizes on the outcome traits.

Multiple regression analyses were performed to assess the independent association between *FTO* genotypes and each specific outcome. A systematic approach was followed (**Table 5-1**). When obesity-related phenotypes were the outcome, firstly, a univariate model including *FTO* genotype only was run (base model). Each of the covariates and categorical factors of interest (age, sex, country and mean valid time of the accelerometer being worn) were then added individually to the base model, to test whether the main genotype effect differs by these factors. Then, all covariates and factors were simultaneously added to the base model. Finally, interaction terms were included one at time (these tested the interaction between categorical variables and *FTO* genotype: *sex* FTO* and *PA*FTO*). These interactions tested whether the association between *FTO* and obesity-related phenotypes differed by sex and by amount of physical activity or sedentary behaviours. Physical activity was fitted into the model as a categorical variable using age and sex specific tertiles for all subcomponents of physical activity (sedentary time, MVPA and Overall PA).

To determine the genotypic odds ratios of being overweight and obese, a logistic regression was used. Obesity status was coded based on Cole et al. cut off points and *FTO* was coded as additive (0, 1, 2). This analysis was further adjusted for age, sex and country.

Table 5-1 Model building approach.

Models	Variable in model (outcome = Obesity-related phenotypes and PA)
base model	Outcome = *FTO* genotype
Model 1	Outcome = *FTO* genotype + covariates $_{\text{(added individually)}}$ ¥ ŧ
Model 2	Outcome = *FTO* genotype + covariates $_{\text{(added simultaneously)}}$ ¥ ŧ
Model 3	Outcome = *FTO* genotype * Factors + covariates $_{\text{(added simultaneously)}}$ ¥ ŧ

Outcome= Obesity-related phenotypes. ¥ Covariates: age, sex, country and mean valid time the accelerometer was worn.

The *FTO* genotype*Physical activity interactions were examined by creating categorical activity variables representing tertiles of each measure, which were included in the models using a GLM

approach. For overall PA, a binary variable was derived to classify children as active (583> min.day) and inactive (<583 Min.day). This cut-off was derived from the 50th percentile of the overall PA variable. All analyses were performed using Statistica (version 8.0; StataSoft, Tulsa, USA) and STATA (version 11.0; College Station. Texas. USA). For all analyses statistical significance was defined as $p<0.05$.

5.4 Results

A total of 4407 children were genotyped for rs9939609 in the *FTO* gene. The minor allele frequency (MAF) of rs9939609 was 0.40 in the whole sample in accordance with expectations for population samples of European origin (Frayling et al. 2007). No significant differences in the genotypic distributions were found between boys and girls (MAF: boys = 0.41; girls = 0.40; p = 0.392). Similarly, no significant difference in the genotypic distribution where found between countries (Chi-Square= 13.017, DF= 7, P-Value= 0.192).The genotype distribution was within Hardy-Weinberg equilibrium in each national sample (Table 5-2) and within the whole population as well, suggesting that ancestry selection proxies that we used were adequate (Frayling et al. 2007).

Table 5-2 *FTO* rs9939609 genotype frequency by country of the IDEFICS study.

Italy	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	176	31.1	55	0.601	0.438
	AT	271	47.9	---	---	---
	AA	119	21.0	45	---	---
Estonia	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	181	31.9	55.8	0.544	0.461
	AT	271	47.8	---	---	---
	AA	115	20.3	44.2	---	---
Cyprus	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	201	40.8	63.1	0.886	0.352
	AT	220	44.6	---	---	---
	AA	72	14.6	36.9	---	---
Belgium	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	229	42.5	63.9	2.710	0.099
	AT	231	42.9	---	---	---
	AA	79	14.7	63.1	---	---
Sweden	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	198	36.2	59.1	1.397	0.237
	AT	251	45.9	---	---	---
	AA	98	17.9	40.9	---	---
Germany	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	119	23.4	53.4	20.415	0.233
	AT	303	59.7	---	---	---
	AA	85	16.7	46.6	---	---
Hungary	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	192	35.0	60.4	2.124	0.145
	AT	279	50.8	---	---	---
	AA	78	14.2	39.6	---	---
Spain	Genotyping	N counts	Genotype Fq (%)	Allele Fq (%)	Chi-Sq	P-Value
	TT	206	36.9	60.1	0.508	0.476
	AT	260	46.5	---	---	---
	AA	93	16.6	39.9	---	---

X^2 test, Hardy-Weinberg equilibrium was accepted at >0.05. The ancestral allele for this SNP is **A**.

5.4.1 Association of FTO Genotype and Obesity-related Phenotypes

All subjects were initially tested for each of the outcomes to establish the influence of the *FTO* variant on obesity related traits. The unadjusted analyses revealed that the rs9939609 polymorphism was significantly associated, using an additive genetic model for each extra copy of the risk allele (A), with increased body weight [0.529 kg increase per allele (SE: 0.143); p< 0.0001], BMI [0.219 unit (SE: 0.046) p< 0.0001], waist circumference 0.563cm (SE: 0.136); p<0.003], skinfold subscapular [0.230 mm (SE: 0.065); p<0.0001] and skinfold triceps [0.339 mm (SE: 0.081); p<0.0001], as also shown in Figure 5.1.

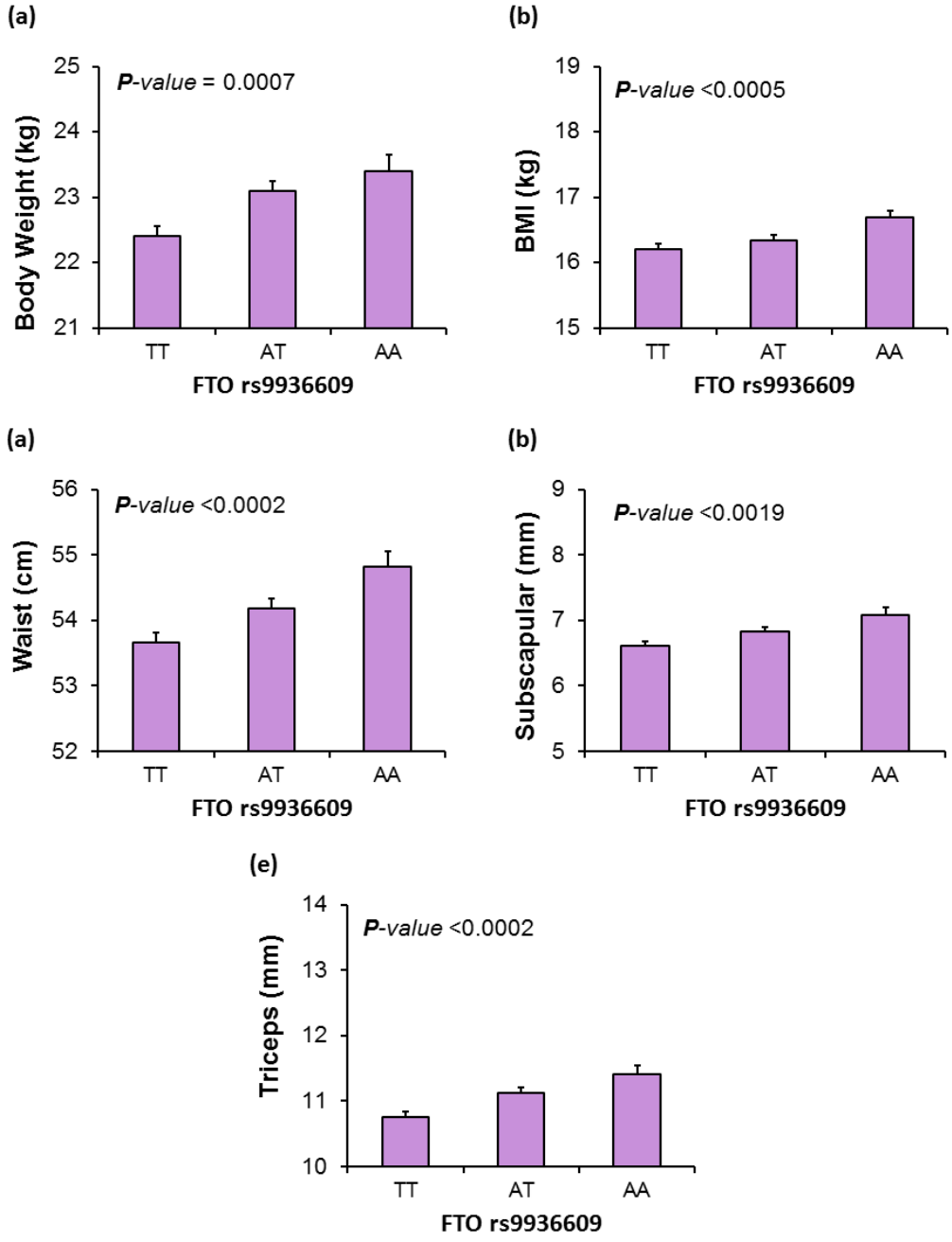


Figure 5.1 Association between rs9939609 and obesity-related phenotypes.

Unadjusted mean and SEM are presented for each genotype group. Linear regression was used to examine the main FTO genotype effect on each obesity-related trait, under an additive genetic model.

To determine whether the *FTO* effect on the obesity-related phenotypes was independent of other confounders, several covariates were included into the model (age, sex, country). Prior to that, it was examined whether the *FTO* rs9939609 genotype effect on the obesity traits differed by sex and therefore an interaction was fitted into the model. There was no evidence of significant interaction detected between *FTO* and sex, [body weight p= 0.658, BMI p=0.845, waist circumference p=0.696, subscapular p=0.191 and triceps p= 0.339] and therefore only the main effect of sex was assessed in the models. After adjustment for all of these confounding factors, the association between *FTO* and obesity-related traits appeared at a lower effect; however it remained significant for all outcomes (body weight 0.382 kg per allele, p<0.0001; BMI 0.119 unit, p<0.0001; waist circumference 0.462 cm, p<0.0001; subscapular 0.189-mm, p=0.003; and triceps 0.293-mm, p<0.0001) (Table 5-3).

Table 5-3 Simple regression coefficients for the association between *FTO* and obesity phenotypes in children of the IDEFICS study.

Predictors	β (95%CI)	Standardised βeta (SE)	p	Adj. r^2
Weight (kg)	0.382 (0.204 to 0.559)	0.040 ± 0.090	<0.0001	0.0016
BMI (kg/m2)	0.199 (0.111 to 0.287)	0.065 ± 0.045	<0.0001	0.0042
Waist circumference (cm)	0.462 (0.240 to 0.684)	0.113 ± 0.051	<0.0001	0.0026
Sub-scapular (mm)	0.189 (0.066 to 0.312)	0.044 ± 0.062	0.003	0.0020
Triceps (mm)	0.293 (0.140 to 0.446)	0.055 ± 0.078	<0.0001	0.0031

Data showed as β coefficient and 95%CI and standardised beta coefficient for each of the outcome. Variables were adjusted for age, sex and country.

The odds of becoming overweight or obese were examined using logistic regression. One copy of the risk allele (A) was not significantly associated with an increase in overweight/obesity risk of 15% (Table 5-4). Those carrying two copies of the risk allele had significantly increased odds of overweight/obesity to 48% after accounting for age, sex and country.

Table 5-4 Odd ratios of being overweight/obese by *FTO* alleles in children.

No of FTO Risk alleles	Model 0 OR (95%CI)	P-value	Model 1 OR (95%CI)	P-value
1	1.17(0.982 to 1.389)	0.079	1.15(0.960 to 1.364)	0.131
2	1.54 (1.239 to 1.913)	<0.0001	1.48(1.185 to 1.842)	0.001

Data presented as OR (95%CI). The obesity status was determined using Cole cut off point, overweight and obese were combined and normal weight was used as a reference group.
Model 0, unadjusted.
Model 1, adjusted for age, sex and country.

5.4.2 *Association of* FTO Genotype and Obesity-related Phenotypes by age categories

To explore how the relationship between *FTO* gene and obesity-related genotypes changes across age categories, a stratified analysis was performed. Table 5.5 shows that regression coefficient has a tendency to increase with increasing age for weight, BMI, and triceps skinfold. However, this was not observed for waist circumference and sub-scapular skinfold. *FTO* was significantly associated with weight and waist circumference in the age category of 6 and 8. BMI was significantly associated with the age category of 7 and 10. Sub-scapular was significantly associated with the age category of 5 and tricep at age group of 5, 6 and 8.

Table 5-5 Age stratified regression coefficients for the association between *FTO* and obesity phenotypes in children of the IDEFICS study.

	Age category <4	Age category 5	Age category 6	Age category 7	Age category 8	Age category <10
n	455	658	507	507	1041	698
Weight (kg)	0.176 (-0.20 to 0.374)	0.092 (-0.175 to 0.359)	0.445 (0.091 to 0.819) *	0.456 (-0.004 to 0.916)	0.443 (0.002 to 0.884)*	0.542 (-0.835 to 1.168)
BMI (kg/m2)	0.086 (-0.046 to 0.219)	0.143 (-0.021 to 0.307)	0.182 (-0.020 to 0.385)	0.241 (0.006 to 0.476) *	0.206 (-0.002 to 0.415)	0.289 (0.004 to 0.573) *
Waist circumference (cm)	0.247 (-0.074 to 0.570)	0.272 (-0.134 to 0.679)	0.355 (0.089 to 1.065) *	0.603 (0.0005 to 1.206) *	0.571 (0.004 to 0.573) *	0.441 (-0.303 to 1.186)
Sub-scapular (mm)	-0.0001 (-0.148 to 0.148)	0.189 (0.015 to 0.363) *	0.139 (-0.89 to0.369)	0.282 (-0.030 to0.596)	0.158 (-0.121 to 0.438)	0.327 (-0.184 to 0.733)
Triceps (mm)	-0.106 (-0.366 to 0.153)	0.330 (0.039 to 0.621) *	0.371 (0.039 to 0.704) *	0.224 (-0.153 to 0.602)	0.377 (0.025 to 0.729) *	0.390 (-0.111 to 0.892)

Data presented as beta coefficients and 95% CI. P-values for differences within ages are given for sex and country-adjusted data. Significance values was denoted as *p<0.05.

5.4.3 Association between *FTO* and Physical Activity-related domains

This section investigates the influence of different physical activity domains (Sedentary time, MVPA, Vigorous time, Overall Physical activity time) on the associations of obesity phenotypes (weight, BMI, waist, skinfolds) and *FTO* gene.

5.4.4 *FTO* and Sedentary Time Interaction: Its Influences on Obesity Phenotypes

Initially, a regression analysis was performed using sedentary time as an outcome to test whether *FTO* influences sedentary behaviours. However, no association between *FTO* rs9930609 and time spent in sedentary activity was found (p= 0.763). To test the occurrence of an interaction effect between *FTO*, tertiles of sedentary time and sex on obesity-related phenotypes, a three way interaction was examined, but there was no significant interactions in all obesity traits [body weight p= 0.903, BMI p= 0.850 waist circumference p= 0.824, subscapular p= 0.884 and triceps p= 0.589]. Subsequently, sex was removed from the interaction to examine the *FTO**sedentary time interaction. However, the interaction (*FTO**sedentary time) was not statistically significant for any of the outcomes (weight =*0.240*; BMI, *p*=0.547; waist, *p*=0.789, subscapular; p=0.304 and triceps, p=0.059) as shown in figure 5.2. After adjusting the model for age, sex and country the interaction remained non-significant. Although no interactions were found between *FTO* genotype and time spent in sedentary behaviour, the effect size of each copy of the risk allele (A) on obesity-related phenotypes increased with increasing time spent in sedentary behaviours. A significant genotyping effect size was found for body weight, however it appeared to be borderline significant for BMI, sub-scapular and triceps skinfold. (Data shown in Table 5.6).

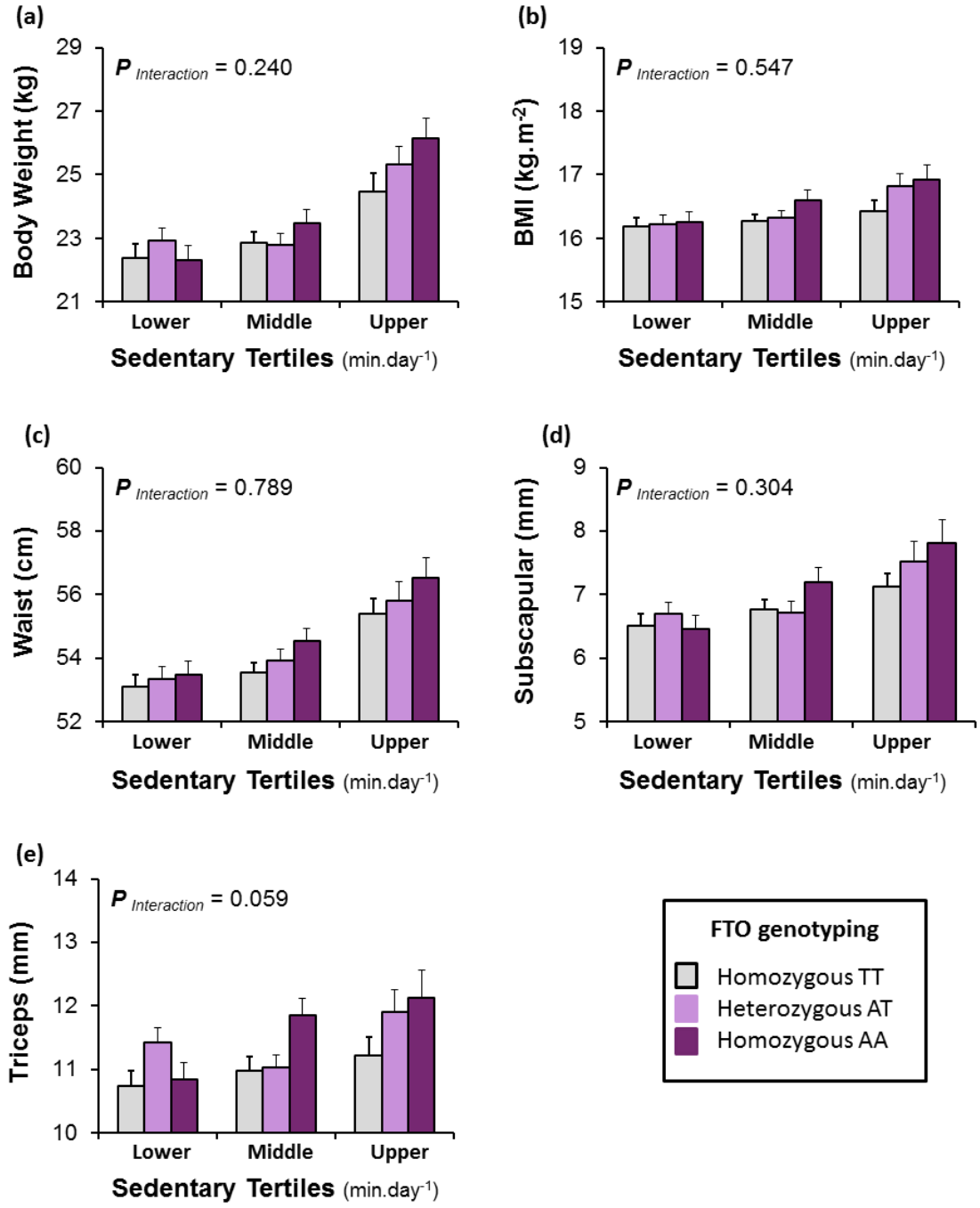


Figure 5.2 Effect of the interaction between rs9939609 and sedentary behaviour on obesity-related trait.

Unadjusted mean and SEM are presented for each genotype group across age and sex-specific tertile of sedentary time (lower, middle, upper) expressed in minutes per day. GLM was used to examine a *FTO**PA interaction and a PA-stratified regression analysis were performed to determine the *FTO* genotype main effect under an additive genetic model.

Table 5-6 Regression coefficients for the association between obesity traits and *FTO* by tertiles of sedentary behaviour.

Outcomes	Lower tertile β (95%CI)	P	Adj. r^2	Middle tertile β (95%CI)	P	Adj. r^2	Higher tertile β (95%CI)	P	Adj. r^2
Weight (kg)	0.020 (-0.66 to 0.70)	0.954	0.001%	0.414 (-0.28 to 1.11)	0.245	0.2%	0.760 (0.01 to 1.51)	0.044	0.6%
BMI (kg/m2)	0.032 (-0.18 to 0.24)	0.764	0.01%	0.193 (-0.03 to 0.42)	0.099	0.4%	0.251 (-0.01 to 0.51)	0.061	0.5%
Waist circumference (cm)	0.137 (-0.46 to 0.73)	0.655	0.03%	0.632 (-0.03 to 1.30)	0.064	0.5%	0.530 (-0.04 to 0.92)	0.153	0.4%
Sub-scapular (mm)	-0.044 (-0.34 to 0.25)	0.771	0.01%	0.274 (-0.06 to 0.72)	0.106	0.4%	0.331 (0.06 to 0.72)	0.098	0.5%
Triceps (mm)	0.099 (-0.29 to 0.49)	0.620	0.04%	0.508 (0.10 to 0.91)	0.014	0.9%	0.426 (-0.04 to 0.89)	0.075	0.5%

Data showed as β coefficient and 95%CI for each of the outcome. *P* values and adjusted r^2 (%) are reported for each variable. Variables were adjusted for age, sex and country. Age and sex specific tertiles were derived for sedentary behaviour and these were fitted in the interaction between PA and FTO gene.

5.5 *FTO* Genotype and Moderate to Vigorous Physical Activity Interaction: Its Influences on Obesity Phenotypes

Initially, regression analysis was performed using MVPA as an outcome to test whether *FTO* influences physical activity behaviours. No association between rs9939609 and Moderate to Vigorous physical activity (MVPA) was found (p=0.587). To test whether the effect of *FTO* on obesity-related phenotypes was modified by levels of time spent in MVPA, a *FTO**MVPA*sex interaction was examined, but there was no significant sex effect [body weight p= 0.768, BMI p= 0.813, waist circumference p= 0.114, subscapular p= 0.566 and triceps p= 0.810] and therefore sex variable was removed from the interaction and was then added as a covariate. Subsequently, the effect of the *FTO**MVPA interaction on obesity traits was examined across the tertiles of MVPA (lower, middle, upper). The interaction was not statistically significant for any of the outcomes (weight =0.700; BMI, p=0.855; waist, p=0.853, subscapular; p=0.444 and triceps, p=0.801) as shown in figure 5.3. After adjusting the model for age, sex and country the interaction remained non-significant. Although no interactions were found between *FTO* genotype and time spent in MVPA, the relationship between each copy of the risk allele and increasing MVPA became significant for those who spent less time in MVPA (lower tertile). A significant genotyping effect was found, in less active children, for BMI, sub-scapular and tricep skinfolds, but it was borderline significant for body weight, and waist circumference. These suggest that the FTO effect on obesity is smaller in children with high MVPA levels and larger in children with less time spent in MVPA (Data show in Table 5.7).

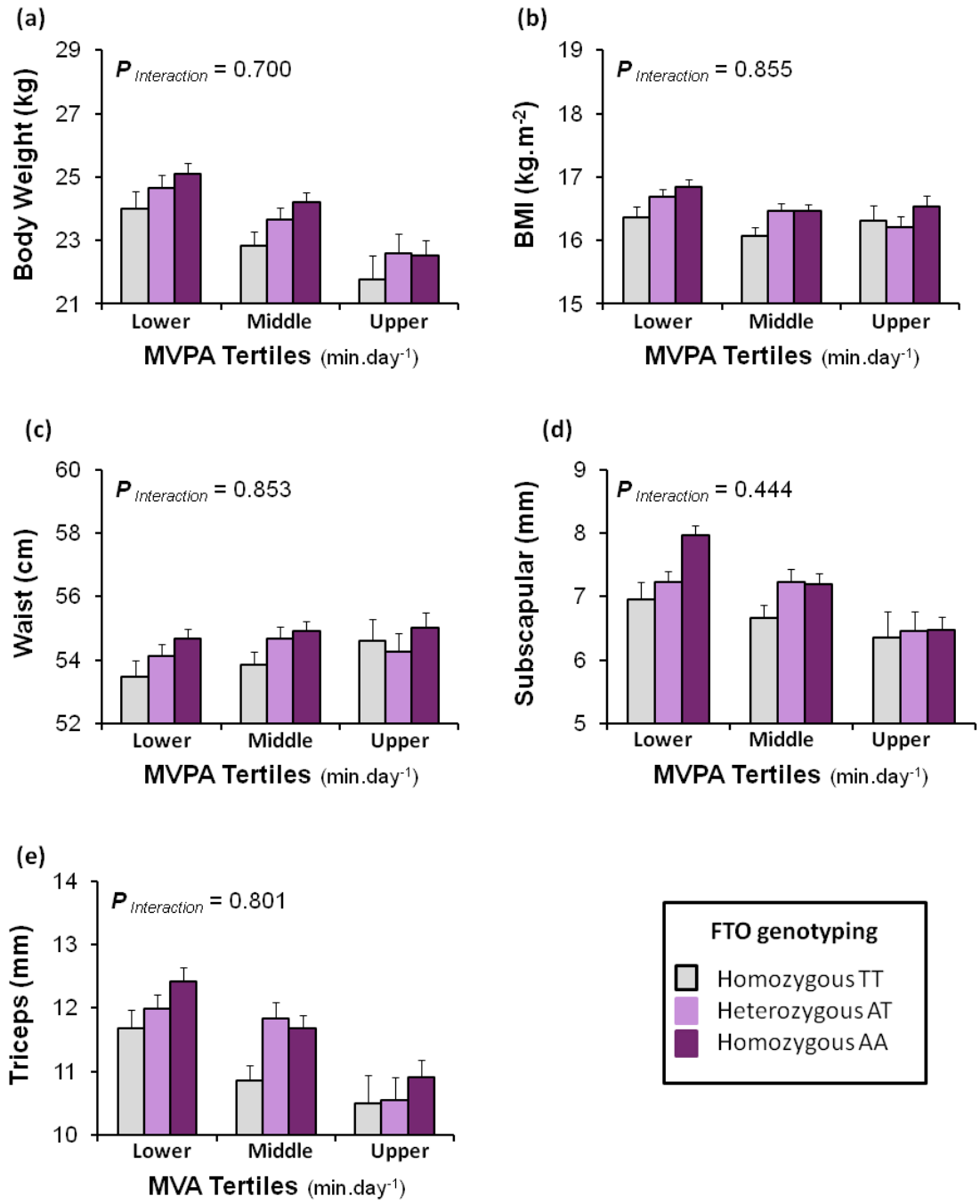


Figure 5.3 Effect of the interaction between rs9939609 and MVPA on obesity-related traits.

Unadjusted mean and SEM are presented for each genotype group across age and sex specific tertiles of MVPA time (lower, middle, upper). GLM was used to examine a FTO*PA interaction and a PA-stratified GLM analysis were performed to determine the FTO genotype main effect under an additive genetic model.

Table 5-7 Regression coefficients for the association between obesity traits and *FTO* genotype by MVPA tertiles.

Outcomes	Lower tertile β (95%CI)	p	Adj. r^2	Middle tertile β (95%CI)	p	Adj. r^2	Higher tertile β (95%CI)	p	Adj. r^2
Weight (kg)	0.715 (-0.11 to 1.54)	0.092	0.4%	0.130 (-0.56 to 0.83)	0.714	0.02%	0.347 (-0.27 to 0.973)	0.276	0.1%
BMI (kg/m2)	0.275 (0.05 to 0.54)	0.045	0.6%	0.099 (-0.13 to 0.33)	0.407	0.1%	0.109 (-0.09 to 0.31)	0.290	0.1%
Waist circumference (cm)	0.742 (-0.04 to 1.53)	0.066	0.5%	0.114 (-0.54 to 0.77)	0.733	0.02%	0.438 (-0.13 to 1.01)	0.135	0.3%
Sub-scapular (mm)	0.433 (0.01 to 0.85)	0.042	0.6%	-0.069 (-0.41 to 0.27)	0.695	0.02%	0.223 (-0.03 to 0.48)	0.088	0.4%
Triceps (mm)	0.542 (0.08 to 1.00)	0.021	0.8%	0.199 (-0.21 to 0.61)	0.351	0.1%	0.335 (-0.55 to 0.72)	0.093	0.4%

Data showed as β coefficient and 95%CI for each of the outcome. *P* values and adjusted r^2 (%) are reported for each variable. Variables were adjusted for age, sex and country. Age and sex specific tertiles were derived for MVPA and these were used in the interaction between PA and FTO gene.

5.6 *FTO* Genotype and Overall Physical Activity Interaction: Its Influences on Obesity Phenotypes

Initially, regression analysis was performed using overall PA as an outcome to test whether *FTO* influences physical activity behaviours. However, no association between rs9939609 and Overall physical activity (Avg_cpm) was found (p= 0.238). To test whether the effect of *FTO* on obesity-related phenotypes was modified by levels of time spent in overall PA, a *FTO**Overall PA time*sex interaction was examined, but there was no significant sex effect [body weight p=0.326, BMI p=0.472, waist circumference p= 0.266, subscapular p= 0.064 and triceps p= 0.082] and therefore sex variable was removed from the interaction and was then added as a covariate. To examine whether the association between *FTO* and obesity-related outcomes is modified by levels of overall PA, age and sex specific tertiles were derived from the total time spent in PA (which include time spent in light, moderate and vigorous physical activities). This variable classified children by level of activity: upper tertile, middle tertile and lower tertile. The *FTO**PA interaction was not significant for all obesity outcomes (weight $p=0.846$ BMI p=808, waist p=0.618, subscapular p=0.161 and triceps, p=0.868) except for BMI, which reached borderline significant interaction (p=0.065) as shown in figure 5.4. Although the interaction was not significant, regression analysis revealed that the association between *FTO* and obesity-related markers was diminished by adopting an active lifestyle (table 5.8). Children that spent less time of overall PA a day showed a significantly higher genotype effect per each risk allele (Weight: 0.883 kg, p=0.042; BMI: 0.375 units, p=0.010; Waist: 0.892 cm, p=0.032; Sub-scapular: 0.466, p=0.038; Triceps: 0.670 mm, p=0.007). Interestingly, the magnitudes of these associations were substantially reduced and became non-significant in children that spent more time in overall physical activity (Weight: 0.044kg, p=0.884; BMI: 0.074 units, p=0.439; Waist: 0.131 cm, p=0.634; Sub-scapular: 0.045, p=0.716; Triceps: 0.173 mm, p=0.0.358). All these associations were independent of age, sex and country.

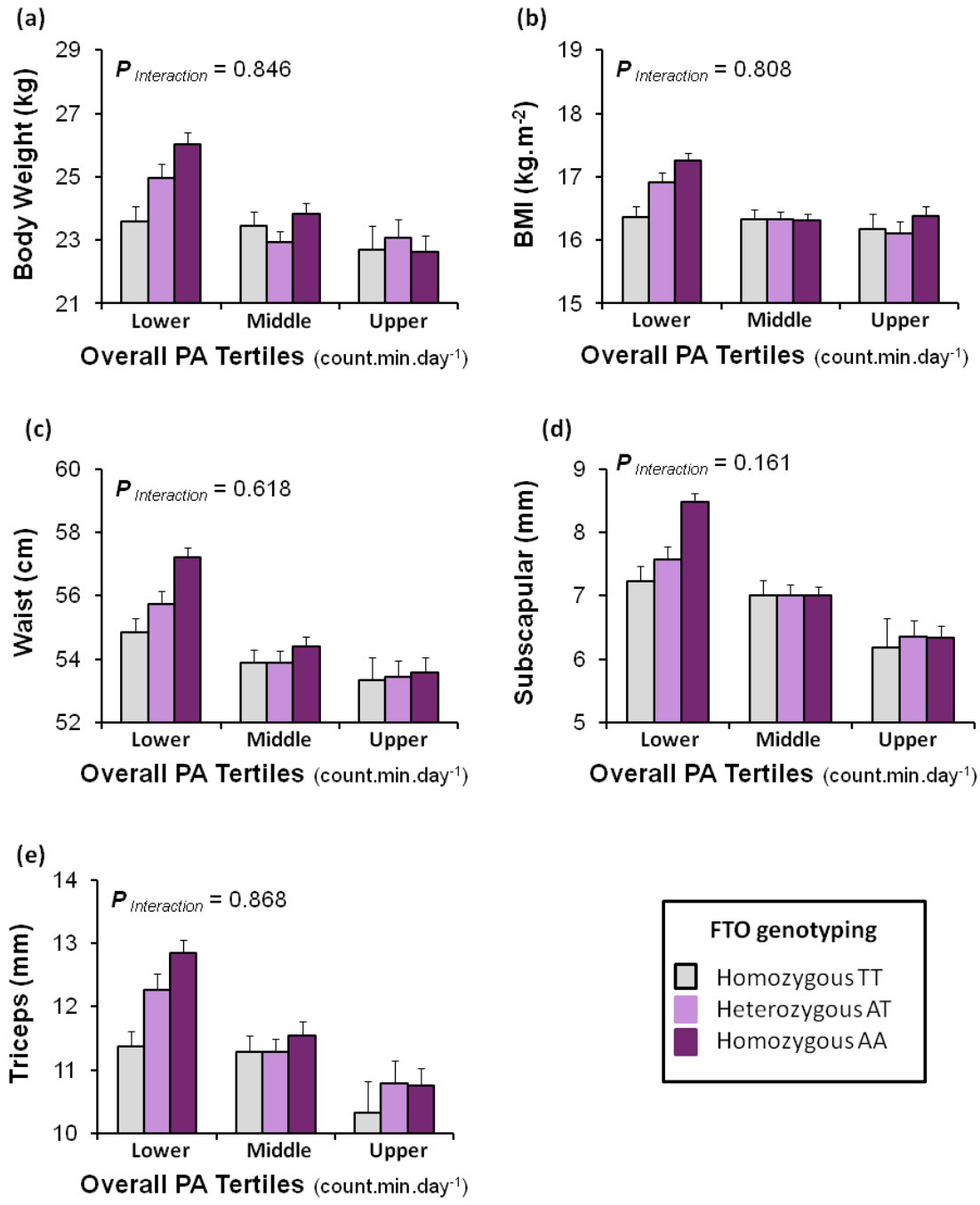


Figure 5.4 Effect of the interaction between rs9939609 and Overall PA behaviour on obesity-related traits.

Unadjusted mean and SEM are presented for each genotype group across the age and sex-specific tertiles of overall PA (lower, middle, upper) expressed in minutes per day. GLM was used to examine a *FTO**PA interaction and a PA-stratified GLM analysis were performed to determine the *FTO* genotype main effect under an additive genetic model.

Table 5-8 Regression coefficients for the association between obesity traits and *FTO* by Overall PA tertiles in children of the IDEFICS study.

Outcomes	Lower tertile β (95%CI)	p	Adj. r^2	Middle tertile β (95%CI)	p	Adj. r^2	Higher tertile β (95%CI)	p	Adj. r^2
Weight (kg)	0.883 (0.03 to 1.73)	0.042	0.6%	0.429 (-0.26 to 1.12)	0.228	0.2%	0.044 (-0.55 to 0.64)	0.884	0.01%
BMI (kg/m2)	0.375 (0.08 to 0.66)	0.011	0.9%	0.080 (-0.14 to 0.30)	0.483	0.07%	0.074 (-0.11 to 0.26)	0.439	0.09%
Waist circumference (cm)	0.892 (0.07 to 1.71)	0.032	0.7%	0.461 (-0.17 to 1.10)	0.157	0.3%	0.131 (-0.41 to 0.67)	0.634	0.03%
Sub-scapular (mm)	0.466 (0.02 to 0.90)	0.038	0.6%	0.149 (-0.18 to 0.48)	0.374	0.1%	0.045 (-0.19 to 0.28)	0.716	0.02%
Triceps (mm)	0.670 (0.18 to 1.15)	0.007	1.1%	0.298 (-0.11 to 0.70)	0.153	0.03%	0.173 (-0.19 to 0.54)	0.358	0.1%

Data showed as β coefficient and 95%CI for each of the outcome. *P* values and adjusted r^2 (%) are reported for each variable. Variables were adjusted for age, sex and country. Age and sex specific tertiles were derived for overall physical activity and these were used in the interaction between PA and FTO gene.

5.7 Discussion

The main findings of this chapter were: a) the genetic variation of the rs9939609 polymorphism of the *FTO* gene was associated with increased obesity traits (measured by weight, BMI, waist circumference or body fat) and; b) although no significant *FTO**PA were detected, this chapter provided some indication that the effect of the *FTO* gene on obesity-related phenotypes could be modulated by lifestyle factors such as physical activity, which could attenuate the genetic effect of rs9939609 on childhood obesity.

The results of this study were consistent with previous findings, which showed clear association between *FTO* variants and body weight (Dina et al. 2007; Frayling et al. 2007). In this chapter, a modest *FTO* genotype effect was found for the combined population (0.529 kg per allele). However, the *FTO* genotype effect size was decreased to some extent after accounting for several confounder factors (0.382 kg per allele), but remained significant. The reduction in the effect size of *FTO* observed in this study after adjustment highlights the need to account effectively for other confounding factors when estimating the strength of genotype effects on body weight.

The association between the FTO SNPs and BMI and the risk of being overweight or obese has been unequivocally confirmed in many studies (Scuteri et al. 2007; Cauchi et al. 2009; Liu et al. 2010). Our results revealed an FTO effect on BMI of 0.219 units per allele in the cohort. However, the strength of this association was reduced after accounting for several confounder factors (0.119 unit per allele), but yet remained significant. In contrast, longitudinal studies have not reported similar results as they did not observe any association of FTO variant with BMI (Jacobsson et al. 2009). In this study, the odds of being overweight or obese were also examined. The analysis revealed that one copy of the risk allele was associated with a 15% risk (not significant) and two copies of the risk allele significantly increased the risk to 48% after accounting for age, sex and country. Similar associations were found in a recent study on Korean children where an association between FTO variants and being overweight was observed (rs9939973: p=0.025, OR=1.47, 95% CI=1.05-2.06; rs9939609: p=0.023, OR=1.53, 95% CI=1.06-2.22)(Lee et al. 2010). In addition, in a different study on children from the TRAILS (TRacking Adolescents' Individual Lives Survey Study) a significant association was found between FTO variant and overweight (odds ratio: 1.34; 95% CI: 1.06, 1.69) at age 16 (Liem et al. 2009). However the PA data was collected with the use of questionnaires instead of an objective measure and therefore results may be less accurate.

The *FTO* variant was significantly and independently associated with body fat, in subscapular (0.230-mm per allele), triceps skinfolds (0.339-mm per allele) and waist circumference (0.563-cm per allele). However, the strength of the association was reduced after accounting for several confounder factors, subscapular (0.189-mm per allele), triceps (0.293-mm per allele) and waist circumference (0.462-cm per allele) but remained significant. A limited amount of evidence has been observed on the effect of *FTO* on waist circumference and body fat in

children, compared to the substantial evidence for BMI. However, previous studies found similar *FTO* associations with adiposity traits (Ruiz et al. 2010). However, the findings of this study and the one in this chapter should be taken with caution due to their cross-sectional nature. In addition, the differences in the genotype size effect between our study and previous studies could be explained due to differences in methods used to estimate adiposity. Furthermore, the inconsistency in the relationship between *FTO* variant and obesity related phenotypes across age groups could be explained by lack of power due to stratification.

According to CDC (Centers for Disease Control and Prevention), children and adolescents should be involved in physical activity for 60 minutes (1 hour) or more per day. Interestingly, there are no recommendations available on reducing time spent in sedentary behaviours. Previously in this section, an effect of the *FTO* gene on obesity traits was described, however genetically predisposed individuals may respond differently depending on their exposure to different lifestyle factors. In addition, it may be hypothesized that environmental influences such as physical activity, may modulate the genetic effect on predisposition to obesity. The literature on the interaction between PA and *FTO* is inconsistent in regards to the levels of PA, the statistical analysis of interactions and the presentation of the interaction results (Palla et al. 2010; Kilpelainen et al. 2011). In our study, although we did not find evidence of an *FTO*-sedentary behaviour interaction, a tendency to increase the effect of *FTO* genotype on obesity traits with increased time spent in sedentary behaviour was observed, even after accounting for several confounder factors (age sex and country); i.e. the effect size per copy of the risk allele for BMI for children with higher time spent in sedentary activities (tertile 3) was 4.2 fold higher, compared to the less inactive ones (tertile 1) (table 5.8). This means that less inactive children could benefit from developing obesity related traits such as increased BMI, whereas less active children may be at risk of developing obesity. However, this finding must be interpreted with caution as the *FTO* gene variant by PA interaction test did not reach significance.

The predominant recommendation regarding public health in order to decrease the risk of obesity and cardiovascular disease, is the increasing time spent in MVPA (Pate et al. 1995). In this chapter, we examined whether time spent in MVPA could modulate the genetic susceptibility to obesity in young children. There was no significant *FTO*-MVPA interaction on obesity traits, and after accounting for age and sex and country, the interaction remained non-significant. Although no interactions were detected, there was some evidence suggesting that children spending more time in MVPA may reduce the genetic effect of FTO on obesity related phenotypes. However, it is important to mention that low levels of MVPA per se and therefore the small variability in this study may be an important factor contributing to lack of interaction. Interestingly, the level of MVPA in children was very low and only a small number of them met the time recommendation on physical activity per day (60 minutes per day of moderate to vigorous PA). This could be justified by two reasons; one related to the adoption of a western lifestyle, which could have a strong effect on reducing physical activity level in children. A second potential explanation is related to the cut-off point used to estimate physical activity

intensities. It is possible that cut-off points used in this thesis were very high for the current level of physical activity on IDEFICS cohort. In addition, taking into account the large number of participants with physical activity data across several countries in Europe recruited in the IDEFICS study, it seems that more appropriate cut-off points are needed for children (Evenson et al. 2008).

The hypothesis whether the association between *FTO* and obesity-related outcomes is modified by the level of overall PA, was derived from the total time spent in light, moderate and vigorous physical activity. This variable was age and sex specific and classified children by level of activity (upper tertile: more active children and lower tertile least active children). Although the *FTO**PA interaction was non-significant for any obesity outcomes, the analysis revealed that the association between *FTO* and obesity-related markers was diminished by adopting an active lifestyle. Children that spent less minutes of Overall PA per day presented a higher *FTO* genetic effect; however these associations were reduced in active children who spent more time in Overall PA per day. This finding could suggest that maybe lower physical activity intensity could still be beneficial for reducing obesity in children. Additionally, considering that most of children's daily time is spent on light intensity activities and only a small proportion on intensities above moderate, it could be highlighted that even physical activity below the recommended intensities could be beneficial for children health. Nowadays, too much emphasis has been given on physical activities above moderate intensity. However, it is important to mention that the level of physical activity within this age range is mainly focused on light intensity activities such as walking. Therefore, in further studies it may be necessary to address whether increasing light intensity physical activity instead of moderate to vigorous physical activity could be a potential strategy to reduce the genetic predisposition to obesity in children.

The results provided in this section are in agreement with the majority of studies that have investigated *FTO* and lifestyle associations; however they come in contrast with other studies which have revealed strong *FTO* and lifestyle interactions such as PA. A recent gene-environment interaction analysis from the Growth, Exercise and Nutrition Epidemiological Study in preSchoolers (GENESIS) cohort, demonstrated a significant *FTO*-PA interaction in males (P=0.007), but not females (P=0.74), and that the *FTO* genotype effect was more pronounced in inactive than active males (Scott et al. 2010). Adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study were tested for a possible gene-PA interaction. They found that the *FTO* rs9939609 polymorphism effect on obesity measures was much lower in adolescents who met the daily physical activity recommendations (i.e., ≥60 min/d of moderate to vigorous physical activity) compared with those who did not (Ruiz et al. 2010). Additionally, during previous studies in children, physical activity did not appear to modify *FTO* genotype on obesity associations (Liem et al. 2009). Similarly, in the Liu et al. study, although they found strong associations between the *FTO* variant and obesity related traits, physical activity was not associated with the *FTO* genotype in European- and African-American youth (Liu et al. 2010) Identifying genetic and environmental interactions is difficult as it requires large sample sizes

and is also challenging to measure accurately lifestyle exposures which may reduce statistical power (Smith and Day 1984; Wong et al. 2003).

A strong asset of the present study is the use of precisely standardized phenotypic measurements within the eight European countries participating in the survey. In fact, all measurements were conducted according to detailed standard operation procedures. In addition, in the present study, the genotyping strategy was part of the study protocol and was agreed before the study commenced. This allowed the extraction from the whole IDEFICS population of a country-, sex- and age-balanced random sample of children, whose size was *a priori* defined, in order to have enough power to detect biologically and clinically relevant differences in the main obesity-related outcomes.

However, there are some limitations that should be taken into account when interpreting the results and findings of this study. Although, in the present study, the genotyping strategy was part of the study protocol, the gene and environment interaction was not the main aim of the project. Despite this, the IDEFICS cohort provided at least 90% power to detect a 20% difference between AA and TT homozygotes in obesity-related phenotypes at a significance level of $\alpha = 0.05$, assuming an additive mode of inheritance (Lauria et al. 2012). The power calculation was not based on a gene*environment interaction, and for this reason, the main conclusion of this chapter regarding gene and PA interaction must be interpreted with caution. This study showed a small effect of physical activity-related factors on the relationship between *FTO* and obesity measured for the IDEFICS population. However, the lack of interaction could be explained from lack of power, as only half of the cohort with genotypic data provided objectively measured PA data. This could also explain why the interaction effect between *FTO**PA behaviour on obesity-related phenotypes was non-significant. Unfortunately, not all children with genotyping data had PA data, and this reduced substantially the number of people included in these interaction models. Further studies should take this into account and aim for higher sample sizes, where both PA and genotyping data are available in the same data set. A second factor to consider is the variability in age and sex, which could confound some of these findings, although age and sex specific tertiles were used in the analysis in order to decrease the possibility of bias. Other factors, which could affect and modify these findings, are related to cultural and socio-economic differences within countries. Although country was added as a covariate in the models, there are still other confounding effects that were not taken into account. Future studies should aim to collect more data related to other lifestyle and social factors that could have important contribution to modulating this relationship between FTO and obesity.

In conclusion, bearing in mind the cross-sectional nature of this study which does not allow conclusions to be drawn about the causality of these interactions, our findings were: 1) this study confirmed and expanded the evidence on the influence of *FTO* on obesity-related traits in children from European countries; 2) although there was no evidence of a significant PA**FTO* interaction, further studies should explore the potential effect of increasing total physical activity on reducing time spent in sedentary behaviour, could be beneficial for reducing the

genetic effect of FTO on obesity related traits in children; this study could have an important public health value in explaining whether PA could be a tool for reducing the genetic predisposition to obesity traits induced by variations in the *FTO* gene; 3) finally, we suggest that further studies are needed in order to understand the mechanisms underpinning the genetic predisposition to childhood obesity. This will aim for more efficient design and implementation of lifestyle strategies to reduce metabolic risk in different populations and for advancing the basic understanding of the mechanisms underpinning human obesity in young children.

6 General Discussion

Throughout the chapters of this thesis, a number of research questions have been considered related to the influence of environment and lifestyle factors on obesity in children from eight European countries. First, the effect of the environment on obesity related phenotypes was determined in European children; second, the influence of lifestyle such as physical activity related factors on childhood obesity was investigated in the IDEFICS population; and third, the effect of the *FTO* rs9939609 polymorphism on obesity related phenotypes and physical activity was examined. Finally, the influence of lifestyle factors such as physical activity patterns on the association between *FTO* variant with obesity was investigated. This thesis focused particularly on European children between the ages of two and ten years old and aimed to investigate the genetic and environmental factors related to childhood obesity.

The main findings described in this thesis were: (a) that age is an important factor when studying childhood obesity, as body composition changes in a significant way with increasing age in both sexes; (b) that environmental and lifestyle effects such as PA on childhood obesity differ between the two sexes and among age groups; (c) MVPA is significantly associated with adiposity related markers independent of time spent in sedentary behaviour; (d) the risk of becoming overweight/obese was lower on children within the middle MVPA tertile and even lower on those with higher levels of MVPA (upper tertile), compared to children that were less active; e) the genetic variation of the rs9939609 polymorphism of *FTO* gene was associated with increased obesity traits (measured by BMI, weight, waist circumference and skinfolds); f) After adjustment for other confounders (age, sex, country), the association between *FTO* and obesity-related traits diminished but still remained significant g) the odds of becoming overweight or obese was examined. The analysis revealed that two copies of the risk allele significantly increased the risk to 48% after accounting for age, sex and country). Overweight and obesity are now considered global threats to health worldwide and have been constantly growing over the years; the obesity epidemic had progressed rapidly in children and adolescents in most countries of the developing world (Kalies et al. 2002; Lobstein et al. 2004). According to WHO, obesity rates have doubled in the past 30 years worldwide (WHO 2011). During this time childhood obesity increased substantially in almost all industrialized countries and in several lower-income countries. Between the 1970s and late 1990s, rates of childhood overweight and obesity more than doubled in many countries (Wang and Lobstein 2006). In addition, while the highest rates of childhood and adult obesity are observed in developed areas of the world such as North America and Western Europe, developing countries are facing rising rates of obesity and consequently increased incidence of non-communicable disease (Amuna and Zotor 2008). In Britain, the average recorded energy intake has declined substantially as obesity rates have escalated,

indicating that physical inactivity may influence the increase of obesity prevalence (Prentice and Jebb 1995).

Furthermore, the rising prevalence of obesity represents a global public health issue; with an estimated 30% of coronary heart disease (CHD) and ischemic stroke and almost 60% of hypertensive disease in developed countries, attributing to excess body mass index (BMI) (WHO 2002). The rising prevalence of obesity in children is of particular concern since 32% of children and adolescents in the United States belong to or are above the eighty-fifth percentile of the 2000 BMI-for-age growth charts (Kuczmarski et al. 2000; Ogden et al. 2008). In addition, according to the 2004 figures of the British Heart Foundation (BHF 2008), United Kingdom shows prevalence of obesity in children aged between 5 to 17 years to be 29%. Studies show that adiposity has been shown to pursue from childhood into adult life, potentially increasing the risk associated with adult obesity (Serdula et al. 1993; Singh et al. 2008). The relationship between child and adult obesity is quite complex, and taking into consideration that persistence of obesity is related to gender and age, designates this association even more challenging. In addition, the strength of the relationship is dependent on the obesity defining criteria used (Wright et al. 2001). Serdula et.al confirmed that for all studies and across all ages, the risk of adult obesity was at least twice as high for obese children compared to non-obese children, and about half (42 to 63%) of obese school-age children were obese as adults (Serdula et al. 1993). However, the limitation with prevalence data in children is compounded by the lack of consensus on obesity definitions, which may result in underestimations. There is a large amount of literature suggesting high prevalence of adiposity in young children, however, the importance of age must not be underestimated when studying overweight and obesity trends in children. As body composition changes in a significant way with increasing age, it becomes more challenging when trying to estimate the rates of obesity in children. Manios and his colleagues tried to examine the relationship between age and 20-year changes on anthropometric characteristics of Greek boys, and concluded that body weight and BMI were higher in 2002 than the 1980s, and this held true for all age groups (Magkos et al. 2006). Additionally, bodyweight and BMI were higher now than in the 1980s, and this was true for all age groups ($p < 0.001$). Increases in weight also showed a decline with advancing age (+17.4%, +13.9%, and +4.0% for 9, 12, and 15 year olds, respectively), whereas BMI changes were similar to those of 9 and 12 years old (approximately 10.5%), but were almost 2-fold higher than in 15 year olds (+5.5%). This again confirms the increased anthropometric and obesity related variables with increasing age and over the years. In this thesis, a visible and significant trend of increased anthropometrics and obesity-related phenotypes, with increasing age in both boys and girls for unadjusted and adjusted analysis was observed. This relationship started to become more evident only after the age group of seven (from six to ten years old). This finding suggests that both age and body composition play a key role when studying obesity on children, and studies with a wide age range should be able to better extrapolate variations in anthropometric characteristics due to age differences.

In addition, a large contemporary cohort of English children, (the Avon Longitudinal Study of Parents and Children, ALSPAC; children born between 1991-1992) found that the incidence of obesity was highest during mid-childhood (age 7-11 years old, 6.7%) and slightly lower during early childhood (3-7 years old, 5.1%) (Hughes et al. 2011). These data are in agreement with the findings of this thesis. Previous research in healthy children and adolescents found girls to have on average 3.8% more fat than boys at the age of 5 which increased to 12.9% by the age of 18 (Shaw et al. 2007); suggesting a role of pubertal stage in body fat. Adding to this, puberty is known to affect body composition with increases in all aspects of body composition (fat mass, lean mass etc.) observed during the period of pubertal growth and development (Siervogel et al. 2003). The Growth, Exercise and Nutrition Epidemiological Study in preSchoolers (GENESIS) which evaluated the food and nutrient intakes, as well as growth and development of a representative sample of Greek toddlers and preschool children, confirmed the high prevalence of overweight children in the preschool population (Manios 2006). Considering that obesity is difficult to treat and prevent, childhood and adolescence are key periods for interventions to set individuals on a healthier lifestyle. This study reported Italy to have the children with the highest obesity related anthropometric characteristics (BMI, waist and hip circumferences subscapular and triceps skinfolds) in both sexes compared to children from other countries. This might be due to specific phenotypic characteristics of the area in Italy where the data was collected, or due to country related environmental factors related to dietary and physical activity behaviours.

In addition to the population characteristics, physical activity is an essential factor to consider when trying to treat or prevent obesity. The role of physical activity in the prevention of disease and premature mortality was first discovered during the 1950's, when it became apparent that the incidence of coronary heart disease (CHD) of London bus drivers was much higher than that of bus conductors (Morris and Heady 1953). It is commonly accepted that decreased levels of PA result in being overweight and obese (Ebbeling et al. 2002). Given the limitations of self-report methods and the high cost and participant burden related to other objective methods (e.g., HR monitoring, doubly Labeled water), accelerometry has become the method of choice for measuring physical activity in free-living children and adolescents (Trost 2001; Troiano 2005; Troiano et al. 2008). The use of accelerometers is now considered an objective way of measuring physical activity in both children and adults. However, the impact of methodological decisions on accelerometer data in children has been examined as epoch and cut-off point were found to significantly influence the classification of sedentary and MVPA time and observed compliance to the MVPA guidelines (Ojiambo et al. 2011). In addition, the existence of different cut points of PA levels may significantly hamper estimations of PA levels, generate confusion and make the comparability of results difficult. A very important concern is that the choice of a cut point which is too low would increase the number of inactive children wrongly classified as active, while an inappropriate high cut point would lead to an increase in the number of active children wrongly classified as inactive (Van Cauwenberghe et al. 2010). Therefore, special attention is needed when trying to interpret PA data measured with the use of accelerometry, as the lack of one consensus of PA cut points may make the PA estimation quite challenging. In this study,

objective measures were used for the most efficient estimation of PA patters in European children. PA data was collected with the use of both Actigraphs and Actitrainers along with an activity diary completed by the parents during the days the children wore the device. As opposed to self-administered questionnaires, accelerometers provided more accurate data in a sample of children from the IDEFICS study (n=2012). However, the use of this kind of devices may be challenging when compliance and correct use of the device is taken into account and therefore propped instructions are necessary in order to eliminate error estimations and misinterpretations of PA data.

Interestingly, a large study which aimed to measure fitness performance variation of children across countries, revealed that the best performing children were that of Northern European countries such as Estonia, Iceland, Lithuania, and Finland (0.6 - 0.9 standard deviations above the global average), and the worst performing children were that of Singapore, Brazil, USA, Italy, Portugal, and Greece (0.4 - 0.9 standard deviations below the global average) (Olds et al. 2006). This evidence suggest that there is a global variation in relation to the performance and physical activity of children; however it is important to consider the measure tool of PA and cut points used. In this study physical activity patterns stratified by country showed increased sedentary behaviour, lower MVPA and time spent in overall PA in Italy and Cyprus and less time spent in sedentary behaviours were observed in Belgium, Germany and Hungary. This PA variation within countries may involve several factors such as country specific school systems and local regulations. Therefore, it is important to be conscious when trying to interpret country associations with PA patterns.

However, apart from the country variation, the patterns of PA of children vary between the two sexes and age groups. Data from the Avon Longitudinal Study of Parents and Children (ASPAC) showed higher level of physical activity (counts/min) of 2.3 (95% CI 0.9 to 3.7) in boys and 0.7 (95% CI -0.1 to 1.4) in girls. This suggests that a higher male-typical behaviour during early childhood is associated with higher physical activity during early adolescence, particularly in boys (Mattocks et al. 2010). In this study, a trend to increase MVPA with increasing age was observed in both boys and girls ($p<0.0001$). Both sexes were found to spend more time in MVPA when being older (>6 years) compared to those younger than 6 years. The time spent in overall physical activity showed a trend to decrease with increasing age in boys ($p=0.0002$) but no significant trend was observed for girls ($p=0.292$). In addition, the environment in which children are raised seems to be an important factor when trying to study and explain children's activity habits. A very recent study revealed a modest but significant impact of built environment on the physical activity of 596 school children in the study region, supporting the potential application of the movability index (Buck et al. 2011). It is therefore essential to consider all the parameters associated with PA in children when designing studies relating to childhood obesity, as age, sex and the environment seem to have an important effect.

In addition to physical activity, the adoption of sedentary behaviour or inactivity may also be related to obesity traits in children. Since sedentary activities involve activities which require minimal exertion from the individuals such as television viewing and video game playing, it may be linked to assumptions that sedentary behaviours are associated to obesity in that they encourage excess food consumption through exposure to advertisements and less physical activity (Gortmaker et al. 1999; Robinson 1999; Wiecha et al. 2006). Although several studies have shown independent associations between physical activity and sedentary time, there is very limited data on the combined effect of these variables on childhood overweight and obesity. Evidence from the Youth Risk Behaviour survey (YRBS) in the US, found that children not meeting the PA or TV viewing recommendations were 3 to 4 times more likely to be overweight than those complying with both recommendations. (Laurson et al. 2008) Furthermore, those meeting the physical activity and screen time recommendations were the least likely to be overweight. Approximately 10% of boys and 20% of girls who met both guidelines were overweight. In comparison, 35% to 40% of children who did not meet either recommendation were overweight. These results demonstrate the effectiveness of these recommendations with regards to childhood overweight and obesity, as well as the importance of the study of the combined effect of physical activity and sedentary time. However until recently, sedentary behaviours have been measured using subjective, self-reporting proxy techniques and are therefore liable to recall bias. This thesis provided evidence related to sedentary behaviour and obesity traits in European children using objective measures of physical activity and inactivity. The univariate analysis between the obesity measures (BMI, weight, waist, and subscapular skinfolds) and time spent in sedentary time showed significant associations, however tricep measure was nominally associated to sedentary time. However, when the models were adjusted for moderate to vigorous physical activity (MVPA), associations between sedentary behaviours and BMI, weight, subscapular and tricep were abolished, indicating that the relationship between sedentary behaviours and obesity-related phenotype is not independent of MVPA and those children that spent more time in sedentary activities could reverse the detrimental effect of sedentariness by increasing their time spent in MVPA. Evidence related to overweight/obesity prevalence in European children and the association of PA was also provided. The risk of overweight/obese was 20.9% lower on those within the middle MVPA tertile and 42.2% on those with higher levels of MVPA (upper tertile) compared to children that were less active (lower tertile). These findings may relate in a way with the findings of a large longitudinal study (ALSPAC study) (Mitchell et al. 2009) in which the minimally adjusted odds ratio of being overweight was 1.18 for every hour spent sedentary in 12 year old children. These findings are in contrast to a different study involving a sample of European children, which reported that positive association between sedentary behaviour (television viewing) and adiposity was independent of physical activity and other confounding variables (Ekelund et al. 2006). Differences in findings between the two studies may be due to self-reported television viewing hours being used as a proxy measure of sedentary behaviour, the use of BMI as a measure of adiposity or the adjustment for total physical activity and not specifically MVPA (Ekelund et al. 2006). In general, the literature supports a need to specifically limit time spent engaged in sedentary behaviour in order to avoid

excess adiposity. Furthermore, there is a lot of attention to prevent decreased physical activity related disorders such as obesity, focused on interventions aimed at increasing time spent in MVPA activities. National and local regulators should reinforce and encourage strategies to increase physical activity behaviour and eliminate sedentary lifestyle in youth, thus decreasing the risk of obesity related diseases.

Like many others, we have shown associations between lifestyle factors such as PA, adiposity and obesity in children of European descent. Since the genetic background has not changed during the past decades, it becomes more evident that the rapid increase in childhood obesity during the recent decades must be attributed to changes in environment and lifestyle factors rather than genetic influences. However, it is believed that genetic background may determine the way we respond in a specific environment. Evidence of gene-environment interaction in the development of obesity was first provided by descriptive epidemiological studies such as twin and adoption studies that compared the risk of disease between genetically related populations who have adopted different lifestyle (Cornes et al. 2009). In addition, epigenetic mechanisms are believed to have a significant role in the development of diseases and chronic conditions such as type II diabetes and obesity related traits in children.

Genome-wide association studies have identified reliable associations between BMI and adiposity with variations in *FTO* gene both in adult and child populations (Dina et al. 2007; Frayling et al. 2007; Hinney et al. 2007; Scuteri et al. 2007; Price et al. 2008). In addition, the heritability of the BMI/*FTO* association has been examined in a longitudinal study on young children; where it was found that *FTO* is age dependent and that the proportion of variance in BMI explained by shared environment diminished with age (Haworth et al. 2008). Furthermore, the association between *FTO* SNPs and BMI and the risk of being overweight or obese has been confirmed in multiple populations (Loos and Bouchard 2008; Loos 2009; Vimaleswaran et al. 2009; Fawcett and Barroso 2010). During this study the role of genes and specifically *FTO* on its contribution to obesity related traits was examined. In addition how lifestyle factors such as PA could modulate the relationship between *FTO* and the two conditions mentioned above was also explored. Our results confirmed and extended this previous association between *FTO* and obesity-related traits for a young European population.

The association between *FTO* and obesity traits in the population studied in this thesis revealed that the rs9939609 polymorphism was significantly associated with increased body weight, BMI, waist circumference, skinfold subscapular and skinfold triceps using an additive genetic model. After adjustment for all of the confounding factors, the association between *FTO* and obesity-related traits appeared at a lower degree, however remained significant for all outcomes. This highlights the need to effectively account for other predictor factors when estimating the strength of genotyping effect on obesity-related traits. Previous studies in children have reported similar *FTO* effect on adiposity measures (Frayling et al. 2007; Liu et al. 2010; Rzehak et al. 2010). Since Frayling et al (Frayling et al. 2007) first reported the significant associations

between the *FTO* variant rs9939609 and adiposity- related phenotypes such as BMI, weight, waist circumference, %BF and skinfolds in both children and adults, several studies have replicated these findings in Europeans (Do et al. 2008; Grant et al. 2008) and Asians (Chang et al. 2008). Furthermore, Liu et al (Liu et al. 2010) in a sample of European and African American youth, replicated the significant associations of the rs9939609 variant with BMI, weight and waist circumference using an additive model. The per-A allele effect of 0.4 kg/m2 in BMI is similar to the effect that Frayling reported in UK children at the age of 11 years and somewhat higher than that of Finnish children at the age of 14. In addition, it was found that the variance in BMI explained by rs9939609 was 0.24%, which is lower than previously reported ~1.0% (Frayling et al. 2007; Freathy et al. 2008). The overall per-A allele increase in weight (~1.3 kg) and waist circumference (~0.8 cm) observed in Liu et al study was similar to the effects reported by Frayling et al (Frayling et al. 2007)(~1.2 kg and ~1.0 cm, respectively). Similar to Frayling et al (Frayling et al. 2007), it was reported that the sum of skinfolds increased with the number of A allele carried; however, the difference did not reach significance. Differences in findings between studies could be partly due to differences in population characteristics, such as age, gender or ethnic composition, as well as environmental exposures.

It is well accepted that lifestyle is becoming more and more sedentary over the years and children spend less time being physically active in outdoor activities. In addition, genetically susceptible children may be influenced differently in specific environments than genetically protected individuals. In this thesis the relationship between *FTO* and sedentary behaviours and its effect on obesity-traits was investigated. The interaction between *FTO**sedentary behaviour on obesity-related phenotypes was also examined. The interaction was not statistically significant for any of the outcomes. However, although the *FTO* genotyping effect size on obesity-related phenotypes appeared to increase with increasing time spent in sedentary behaviours, there was not significant evidence to support this tendency. We also examined the genotyping effect on obesity markers for those individuals which were more inactive compared to the more active individuals (lower tertile). Those children being categorized as inactive appeared to have a significantly higher genotyping effect per each risk allele; the magnitudes of these associations were substantially reduced and became non-significant in active children. No association between rs9939609 and Moderate to Vigorous physical activity (MVPA) and Overall PA tertile was observed on the obesity phenotypes. These findings agree with the few studies that have investigated the effect of *FTO* and lifestyle interaction on obesity (Lappalainen et al. 2009; Liem et al. 2009; Liu et al. 2010). These studies showed a similar effect of PA on the association between *FTO* gene variants and obesity measures. The lack of interaction of *FTO* and PA in children may be in part due to the weak association of childhood BMI and higher activity levels in children compared to adults (Troiano et al. 2008). Interestingly, evidence led scientists to contemplate that exercise behaviour could be explained by specific genes and is therefore hereditary. For exercise ability, coordinated efforts exist worldwide and successful association has been reported for a number of genes influencing endurance or strength phenotypes, some of which have been replicated in independent samples (Perusse et al. 2003; Rankinen et al. 2004).

However, for exercise *behaviour* no such coordinated effort exists, although the most recent version of this *MSSE* Gene Map included for the first time a new section on this topic (Wolfarth et al. 2005). The evidence of exercise heritability requires a change in the perspective, similar to the one changed from "population-based" intervention strategies to "personalized" intervention strategies (Stubbe 2009) and preventive medicine or interventions in the future. However, large scale gene finding studies are needed in order to start targeting the importance of exercise behaviour.

A major challenge of this new information is its translation into public health and clinical practice and more importantly, in the development of prevention strategies. Since the latest discoveries in genetics and genetic predisposition to complex diseases, scientists have raised hopes in the development of the genetic profile from which it will be possible to predetermine possible at risk individuals, more prone to health related conditions such as obesity. Interestingly, although significant scientific progress has been made regarding the area of genetics and obesity, the risk effects have been very small and this may be causing misinterpretations of the real genetic effects. Sample size has a key role when investigating genetic and environmental factors that may interact and predispose to certain diseases. In this study, the chi-square test performed, revealed no significant differences between the genotyping and non –genotyping group concerning the frequencies in each BMI category group and other phenotypes. This suggests that the genotyping sample was representative of the full cohort, and thus the genetic conclusions of this study may reflect the full IDEFICS population.

In addition, not fully accounting for confounding factors may give a misleading impression of the real genetic effect in the development of obesity, and this is something to be considered when evaluating data and designing studies. One of the advantages of the present study is that thorough adjustment for several confounder factors has been made and a large sample size has been used. This has allowed for the actual effect of the risk genetic variant of *FTO* on obesity measures to be discovered, as was initially planned in the study. This study has provided replication of previous association studies, thus added into role the susceptibility loci towards variation in obesity. Since gene- environment interaction studies demonstrate that genetic susceptibility to obesity is modifiable, scientists believe that convincing evidence of gene-environment interaction, may give individuals the motivation needed to obtain a healthier lifestyle (Kilpelainen et al. 2011). Interestingly, there is evidence that some of the choices for a healthy lifestyle reflect differences in genetic makeup, although potentially in interaction with shared environment (Stubbe 2009). Randomized controlled training trials have clearly shown that regular exercise has a causal effect on mental (Babyak et al., 2000; Moore & Blumenthal, 1998; Steptoe, Edwards, Moses, & Mathews, 1989) and physical health (Berlin & Colditz, 1990). It is possible, therefore, that well-known heritability of many health parameters like depression (Kendler & Aggen, 2001), obesity (Schousboe et al., 2003), thrombosis (Dunn et al., 2004), hypertension (Kupper et al., 2005), diabetes (De Lange et al., 2003), and even cardiovascular mortality (Zdravkovic et al., 2004) may partly reflect the genetic factors causing the adoption

and maintenance of regular exercise behaviour. In that case, isolating the "genes for exercise behaviour" immediately translates to finding genes that contribute to the heritability of mental and physical health.

An additional strength of the present study is the use of precisely standardized phenotypic measurements within the eight European countries participating in the survey. In fact, all measurements were conducted according to detailed standard operation procedures. In particular, subsamples of study subjects were examined repeatedly to calculate the inter- and intra-observer reliability of anthropometric measurements (Stomfai et al. 2011). In addition, physical activity and sedentary time were objectively measured, providing greater validity in these measures than would be obtained from self-report questionnaires (Shephard 2003). In addition, the IDEFICS study size allowed for a better understanding of sex- and age-specific physical activity validation and body composition assessment in young children. Moreover, the involvement of different countries during the data collection of this study, allowed for a better insight of country specific anthropometric and lifestyle characteristics in European children. The multicentre approach might however be seen as a drawback of the study due to potential heterogeneity of the data which might have occurred from county-to-country and centre-to-centre differences or errors during field work measurements. However, this study provided valuable information regarding whether the use of unified analysis approaches for data on physical activity and body composition, are justified not only between age groups and sexes but also in different European countries (Bammann et al. 2011). In addition, it is of tremendous importance to use standardized protocols and the same scientific equipment when conducting a multicentre study and also maintain quality control measures. Furthermore, when measuring PA subcomponents, the use of cut-offs may use heterogeneity due to the different methods used to produce activity and inactivity levels. The cut-offs based on fixed percentages or absolute values appear to cause heterogeneity because of the wide differences in the measurement instruments used to provide the continuous measures of PA (Kilpelainen et al. 2011). Although the most accurate method when measuring PA in different countries is to use specific cut-offs based on national PA data, in practice is very challenging as prevalence estimates may differ in the instruments used and the sample representing the population (Kilpelainen et al. 2011).

Due to the cross-sectional nature of this study, it is not possible to draw firm conclusions about the causality of associations observed. A randomized controlled trial is needed to address this definitively. Furthermore, despite the fact that IDEFICS study was carefully designed and offered a relatively large variety of exposure variables, there were a number of confounding variables that were not possible to be accounted for. In addition this study did not examine potential relationship of PA and metabolic markers, for which some researchers have found strong associations. Another limitation of this study might be of missing data and possible errors which might have occurred during data collection. During measurements and data archiving, possible errors may have caused small changes in the actual findings of this study. Also, the use of 60 sec epoch instead of a smaller one (15 epoch) to measure activity counts may have caused

underestimations and thus inaccuracies as far as the activity patterns of the European children are concerned. Moreover, the lack of nutritional data as an environmental factor attenuating Gene*PA interaction effects may be considered as a limitation, as dietary patterns would have possibly influence the findings of the study. In addition, the use of $p<0.05$ as a cut-off point for significance in all statistical analysis may have increased the chance of type I and II error when multiple tests been conducted. The chance of a false positive could increase when the p-value defining statistical significance is not corrected for multiple testing. However, the main finding of this study shows strong evidence of significance and the most relevant result was far from a nominal or borderline significant p-value.

Apart from identifying the limitations of this study, it is important to mention possible design strategies of the IDEFICS study which could have improved the quality of the results if they were done differently. One of the most important points of study design improvements could have been the more accurate matching of data for the genotyping cohort; that way we could have had the maximum number of data in all variables of interest available. By selecting in advance the cohort to be genotyped, special attention could have been paid in order (for this full cohort) to have data in all variables to be investigated (i.e. PA, dietary, SES, demographic data). As a result, this would allow for a more effective study of the European cohort and thus eliminate misinterpretations due to limited data or power. The lack of evidence supporting significant Gene*PA interactions in this thesis could be explained by the limited sample size with all the data of obesity related variables available. However, the design of the study did not include from its foundation a gene*environment interaction sample calculation. The genetic sub-sample was estimated on a direct association between the risk variant of the FTO gene and obesity-related markers. The actual sample size (n=4407) of the IDEFICS cohort provided at least 90% power to detect a 20% difference between AA and TT homozygotes at a significance level of $\alpha = 0.05$, assuming an additive mode of inheritance. This sample size could be underpowered for detecting a gene*environment interaction. Considering Wung and cols, if poor measures of exposure and outcome are used (e.g. rho >0.4) regarding sample size estimation for gene and environment interaction, then a study size of approximately 151,000 people would be required to detect the interaction with 95% power at a significance level of 10−4 (Wong et al. 2003). Such an interaction could be detected in study samples of under 10,000 people if more precise measurements of exposure and outcome were performed (e.g. rho >0.7). Taking into account that correlation (rho) between BMI and PA variables in the IDEFICS cohort lies between 0.4 and 0.7, then our sample (n=2012) will be unpowered to detect a gene x environment interaction. Although the number of children with PA data was relatively large, the number of children with both physical activity and genetic data was reduced. This could be one of the potential and strongest explanations for lack of interaction. Additionally, another study design strategy that could have offered better data is the use of food frequency questionnaire instead of 24 hour recall to collect the dietary habits of children. The food frequency questionnaire, allows more accurate data to be produced and it is less likely to misinterpret dietary patterns of the individuals studied. More accurate nutritional data could have allowed the Gene*Diet interaction

to be investigated and therefore provide better understanding as to the gene/environment relationship. As far as the physical activity data is concerned, a larger sample with PA data could provide more powered and accurate results regarding the activity or inactivity patterns of the European children. However, this is something that was not considered in the design of the study since its origin.

In the case of future work and a continuation of this thesis contribution, several aspects could be further investigated. Molecular markers such as blood lipids, glucose and their relation to FTO may be investigated. Moreover, the relation of PA on molecular markers when interaction is tested with FTO can be a potential question to be answered. Apart from molecular traits, a future step could have been the analysis of more SNPs which have already been genotyped to investigate their relationship with obesity phenotypes and PA. In addition, the study of different PA cut-offs may be a potential future work of this thesis, as it will allow a comparison of activity cut-offs and will provide insight as to which one is more representative for young children.

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

(a) Physical activity and sedentary behaviours influence obesity related phenotypes in children of European origin. These associations persisted after adjustment for a comprehensive range of potential confounding factors.

(b) The *FTO* rs9939609 variant influences obesity related phenotypes in children of European origin. These associations persisted after adjustment for a comprehensive range of potential confounding factors.

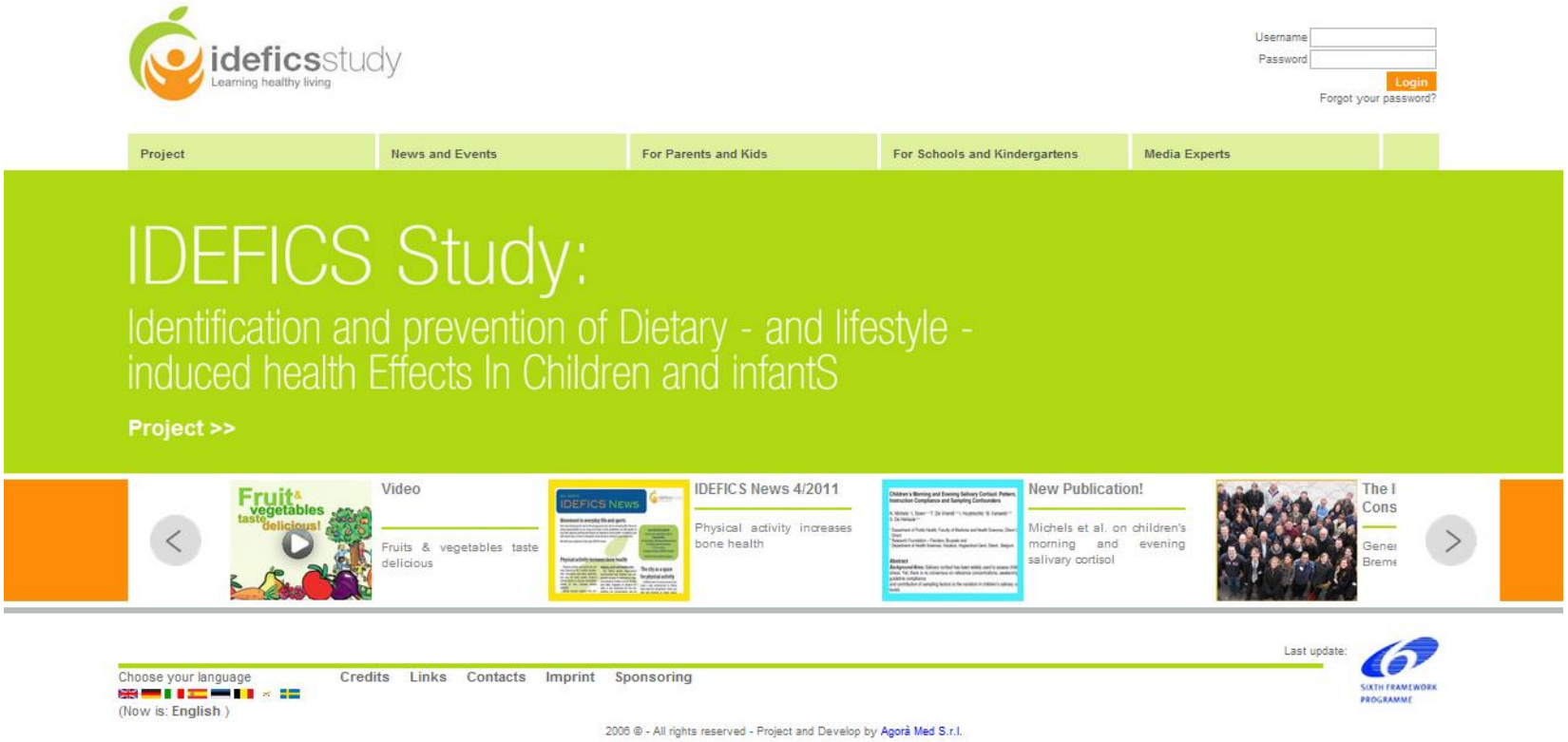
(c) That although there was no *FTO**PA interaction, physical activity and inactivity could play a key role in modulating the genetic predisposition to obesity in children. Further studies are needed in order to address this question, as it has important public health value, since being physically active may have a protective role in the genetic predisposition to obesity induced by variation in the *FTO* gene.

(d) Further studies into the mechanisms underpinning the previous effect are needed in order to more effectively develop accurate design as well as implementation strategies to reduce childhood obesity, and for advancing the basic understanding of the mechanisms behind human obesity and its relationships with genetics.

7 Appendices

7.1 IDEFICS Logo in English

7.2 Snapshot of IDEFICS web page (www.idefics.eu)



7.3 Participants Information Sheet and Consent Form

[*Anonymous ID-number of study subject*]

INFORMED CONSENT FORM

Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infants (IDEFICS)

[*IDEFICS logo, name and logo of local institution conducting the survey*]

Information on the study

The [*name of local survey centre*] is participating in a large European survey to prevent children's health problems like overweight and obesity. The study investigates the eating habits and lifestyle of children, including physical activity and media consumption, as well as their genetic background. It also involves a medical examination of each child.

We invite children who are 2 to 10 years old and who live in [*name of community*]. With this form we would like to ask you for your consent to the participation of your child.

If you agree, you will be asked to complete a parental questionnaire on lifestyle, nutrition and health of your child. We would also like to ask you to agree to a physical examination of your child by a physician. In addition, we will ask your permission to collect biological samples from your child. This includes a mouth swab sample for genetic analyses, a urine sample and a blood sample for the measurement of relevant clinical parameters like glucose, blood lipids or hormones related to energy balance. A set of the most important clinical parameters will be measured immediately and you will be given the information during your visit at the examination site. We would like to get your permission to store possible remaining biological samples beyond the termination of this study. This would enable future analyses of the relationship between genetic factors and the disorders in question.

As the responsible scientist of this national survey within the IDEFICS study, I will guarantee that all personal information, results of examinations and medical records will be treated confidentially. All information concerning your child's participation in the study will only be used for scientific purposes, in accordance with applicable [*national*] law. No one except members of the research team will have access to the answers and test results. All data will be pseudonymised, that means that personal information (including biological samples) will not be labeled with their donor's name, only with an anonymous number. The name of your child will not be used in any report or released in any way. Names and addresses will be kept separately from any survey data under lock and key.

[*City, date*]

_____

[*Name of leading scientist of local survey centre*]

[*Anonymous ID-number of study subject*]

Certificate of consent

I have been informed that my child's participation in this study is voluntary. I am free to withdraw my child from the study any time and without having to give a reason. Choosing not to participate or withdrawing from this study will not result in any disadvantages for me or my child.

I have read this form and the research study has been explained to me. I have been given the opportunity to ask questions. If I have more questions, I know whom to call.

I agree to my child's participation in the study described above. A copy of this consent will be provided to me after I signed it.

I confirm my agreement to the following study components:

Physical examination of my child	yes☐	no☐
Collection of urine sample from my child	yes☐	no☐
Collection of mouth swab from my child	yes☐	no☐
Collection of venous blood from my child	yes☐	no☐
If donation of venous blood is not agreed with:		
Donation of capillary blood from the finger tip earlobe	yes☐	no☐

I would like my child to take part in this research study.

NAME of CHILD (in block letters)

.....

ADDRESS of CHILD (to enable that my child will be contacted again)

ZIP-code:.....City:.....Street:.....

NAME of PARENT / GUARDIAN (in block letters)

.....

To be able to provide you with news we would appreciate to know your Email address (if available): ..... not available☐

SIGNATURE ..... City, Date .....

7.4 Parental Questionnaire

General information

What is your relationship with the selected child?

❍ 1 Biological parent
❍ 2 Adoptive parent
❍ 3 Stepfather or stepmother
❍ 4 Parent in a foster home
❍ 5 Other, please specify: _____

You are:

❍ 1 Male ❍ 2 Female

How old are you?
Please give the ages of parents with whom the child is living.

Mother	Father
|___|___| years	|___|___| years

What is your height and weight?
Please give information for parents with whom the child is living.

	Mother	Father
Height (cm)	|__|__|__|	|__|__|__|
Weight (kg)	|__|__|__|	|__|__|__|

What is the date of birth of the child?
Write day, month, and year.

|___|___| Day |___|___| Month |___|___|___|___| Year

What sex is the child?

❍ 1 Male ❍ 2 Female

How many persons live permanently in the household where your child usually lives?

Number of persons (adults and children): |__|__| persons.
Number of persons below 18 years: |__|__| person(s).

Who does your child live with most of the time?
Please tick the answer that applies most.

❍ 1 With his/her parents

❍ 2 With his/her mother

❍ 3 With his/her mother and her new partner

❍ 4 With his/her father

❍ 5 With his/her father and his new partner

❍ 6 Half of the time with his/her mother and the other half with his/her father

❍ 7 With his/her grandparents or other relatives

❍ 8 With foster parents or adoptive parents

❍ 9 In an institution e.g. orphanage

❍ 10 Elsewhere, please specify: _____

How many older and younger siblings does your child live with?
Count also half brothers and sisters / siblings in-law.

My child lives together with |___|___| older siblings.
My child lives together with |___|___| younger siblings.
My child lives together with |___|___| siblings of the same age.

❍ 0 My child does not live together with any siblings.

Day-care, pre-school and school

Do you currently use a day care service or have a babysitter for your child?

❍ 1 Yes, for approximately |___|___| hours per week.

❍ 2 No ➔➔➔ Please continue with question 12.

Which of the following types of day-care or babysitting do you mainly use for your child? Please tick each that applies.

❍ 1 School day-care service

❍ 1 Other day-care service or centre (including one located in the workplace)

❍ 1 A child's brother or sister baby-sits the child in your home

❍ 1 A relative other than your child's sibling baby-sits the child in your home

❍ 1 A non-relative baby-sits the child in your home

❍ 1 A relative baby-sits your child elsewhere

❍ 1 A non-relative baby-sits your child elsewhere

Is your child ever left on his/her own, for example, before or after kindergarten, pre-school or school?

❍ 1 Yes, approximately |___|___| hours per week.

❍ 2 No

Does your child currently attend kindergarten, pre-school or school?

❍ 1 He/She currently attends kindergarten/pre-school.

❍ 2 He/She currently attends school and attends the |___|. grade.

❍ 3 He/She (still) does not attend kindergarten, pre-school or school. ➔➔➔ **Please continue with question 19.**

❍ 1 My child usually does not eat kindergarten, pre-school or school meals.

Do you regularly give your child money to buy food before, at, or after kindergarten, preschool or school (e.g. for vending machines, vending trucks, kiosks)?

❍ 1 Yes

❍ 2 No

How far is your child's kindergarten, pre-school or school located from your home?

❍ 1 Up to 1 kilometre

❍ 2 From 1 kilometre to 2 kilometres

❍ 3 From 2 kilometres to 3 kilometres

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