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EATING PATTERNS, LIFESTYLE AND MEDITERRANEAN DIET IN CHILDREN AND ADOLESCENTS FROM THE CAMPANIA REGION, ITALY

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CHAPTER 1: INTRODUCTION

'Unhealthy diets and physical inactivity are key risk factors for the major non communicable diseases such as cardiovascular diseases, cancer, and diabetes.' *WHO Global Strategy on Diet, Physical Activity and Health*, 2004

1.1 OVERWEIGHT AND OBESITY IN CHILDHOOD AND ADOLESCENCE: A PUBLIC HEALTH EPIDEMIC

Overweight and obesity are defined as "abnormal or excessive fat accumulation that presents a risk to health". Childhood obesity is one of the most serious public health challenges of the 21st century (Waters *et al.*, 2011). The problem has become global and it does not affect only industrialised countries, but it is increasingly affecting many low- and middle-income countries, particularly in urban setting (WHO, 2004).

Globally, in 2013 the number of overweight children under the age of five, is estimated to be over 42 million. Close to 31 million of these are living in developing countries.

Overweight and obese children will probably become obese adults. This consistently positive associations has been observed already more than 20 years ago by Serdula *et al.* (1993) in a review which showed as about half (42 to 63%) of obese school-age children were obese as adults. For all studies and across all ages, the risk of adult obesity was at least twice as high for obese children as for non-obese children. The risk of adult obesity was

greater for children who were at higher levels of obesity and for children who were obese at older ages.

Obese children and adolescents have higher risk to develop noncommunicable diseases (NCDs), such as diabetes and cardiovascular diseases at a younger age (WHO, 2000).

Because children and adolescents' bodies undergo a number of physiological changes, it is difficult to develop one simple index for the measurement of overweight and obesity in this age range. Depending on the age, different methods to measure a body's healthy weight are available. For children aged 0-5 years the WHO Child Growth Standards, launched in April 2006, include measures for overweight and obesity for infants and young children up to age 5 (Garza and de Onis, 2004). For older children and adolescents WHO developed the Growth Reference Data for 5-19 years (de Onis *et al.*, 2007).

The prevalence of overweight and obesity in adolescents can be defined according to different methods: the main ones are the WHO growth reference for school-aged children and adolescents (overweight = one standard deviation body mass index for age and sex, and obese = two standard deviations body mass index for age and sex) (de Onis *et al.*, 2012) and the extended International Obesity Task Force (IOTF) body mass index cut-offs for thinness, overweight and obesity suggested by Cole and Lobstein (2012).

1.2 OVERWEIGHT AND OBESITY IN CHILDREN AND ADOLESCENTS IN THE CAMPANIA REGION

Italy is one of the European countries with the highest overweight and obesity rate among children and adolescents. Moreover, the prevalence of overweight and obesity in children is not equally distributed geographically: regions from the Centre and from the South (Figure 1.1) have a higher prevalence than regions from the North. Campania is the region with the highest overweight and obesity rate in children (Nardone *et al.*, 2015; Spinelli *et al.*, 2011). These are the results of a national nutritional surveillance system (*Okkio alla salute*) which is promoted and financed by the Ministry of Health of the Italian Government and conducted in collaboration with the Ministry of Education in all Italian Regions.

Okkio alla salute is the Italian response to the need to understand the extent of the epidemic in overweight and obese children. Since 2007, this survey represents an excellent source of epidemiological data on the nutritional habits, lifestyle attitudes and growth status of the child population in Italy.

Italy, with this systematic data collection, participates in the WHO Childhood Obesity Surveillance Initiative (COSI). Interestingly, the same North-South trend, observed in Italy, is also observed throughout Europe. In fact, the countries where the prevalence of overweight and obesity are higher are the Suothern European ones (Greece, Spain, Italy and Portugal) (Wijnhoven *et al.*, 2014).

So far, *Okkio alla salute* have collected data every 2 years (2008-2010-2012-2014). Each data collection involved more than 40,000 children and their parents, and 2,000 schools. The standardised methodology at national level guarantees the comparability of the collected data: weight status, food habits, physical activity, sedentary behaviour of 8-9 year-old children in the familial and school context. Four questionnaires are administered to children, parents, teachers and school directors and anthropometric data of weight and height are measured by trained professionals.

The results from the last *Okkio alla salute* data collection, in 2014, show that, while in Italy 20% of children are overweight and 9.8% are obese (severe obesity is 2.2%), in Campania 28.6% are overweight and 19.2% are obese (severe obesity 5.5%) (Nardone *et al.*, 2015). Possible causes of this discrepancy, apart from individual food habits and sedentary behaviour (which will be illustrated in more detail in chapter 1.3.1), are a smaller number of school refectories, of initiatives for the promotion of physical activity in schools and the lower distribution of healthy foods in schools.

The weight status and the food and lifestyle habits of adolescents were studied by Health Behaviour in School-aged Children (HBSC). The HBSC research network is an international alliance of researchers that collaborate on the cross-national survey of school students. The HBSC collects data every four years on 11-, 13- and 15-year-old boys' and girls' health and well-being, social environments and health behaviour.

The first data collection dates back to 1982, when researchers from England, Finland and Norway agreed to develop and implement a shared research protocol to survey school children. By 1983 the HBSC study was adopted by the WHO Regional Office for Europe as a collaborative study. HBSC now includes 44 countries and regions across Europe and North America. The international standard questionnaire produced for every survey cycle enables the collection of common data across all participating countries and thus enables the quantification of patterns of key health behaviour, health indicators and contextual variables. These data allow to make cross-national comparisons and, with successive surveys, trend data is gathered and may be examined at both the national and cross-national level.

The study group of the Campania region (Mazzarella and Pizzuti, 2011) showed the results of a sample of 1,200 subjects for each age class (11-13-15

year-olds). At 11 years old 30% of the adolescents are overweight and 7% obese. At 13 years old 25% are overweight and 5% obese, while at 15 years old 23% are overweight and 5% obese, with a higher prevalence in males than in females.

1.3 CAUSES OF THIS EPIDEMIC

The principal cause of childhood overweight and obesity is an energy imbalance between calories consumed and calories expended.

Global increases in childhood overweight and obesity are attributable to a number of factors including:

- a global shift in diet towards increased intake of energy-dense foods that are high in fat and sugars but low in vitamins, minerals and other healthy micronutrients;
- a trend towards decreased physical activity levels due to the increasingly sedentary nature of many forms of recreation time, changing modes of transportation, and increasing urbanization (WHO, 2006). In fact, there is a worldwide trend to adopt unhealthy behaviour (Mazzarella and Pizzuti, 2011; Moreno *et al.*, 2010).

WHO recognizes that the increasing prevalence of childhood obesity results from changes in society. The problem is linked not only to children's behaviour but also, increasingly, to social and economic development and policies in the areas of agriculture, transport, urban planning, the environment, food processing, distribution and marketing, as well as education.

The problem is societal and therefore it demands a population-based multisectoral, multi-disciplinary, culturally, and politically relevant approach. Unlike most adults, children and adolescents cannot choose the environment in which they live or the food they eat. They also have a limited ability to understand the long-term consequences of their behaviour. They therefore require special attention when fighting the obesity epidemic.

1.3.1 Eating patterns and lifestyle among children and adolescents

Analyzing in more depth the two components of the energy imbalance, we are going to illustrate how children and adolescents consume their calories and how they spend them. In other words, we will look at food habits, sedentary behaviour and physical activity levels among children and adolescents.

Western Europe has experienced a net increase of about 400 kcal per person per day in the supply of total food energy since the 1960s, much of which can be attributed to the rise in fats and oils, especially vegetable oils, and of course of sugar (Branca, *et al.*, 2007). This increase is not clearly attributable to fats and oils or to sugar alone.

For most countries, fruit and vegetable intake is below recommended levels, which is unfavorable for the energy density of the diet. The increased supplies in some countries may indicate a higher consumption of processed fruit or vegetable extracts, such as juices and syrup concentrates, which usually have a higher energy density than the less processed fresh alternatives. In addition, as fibre intake is also low throughout Europe, consumption of more satiating foods – for example, wholegrain foods – should be encouraged as an additional measure to reduce the energy density of the diet. Sweetened beverages have become common beverages, as shown by the significant increases in the consumption of soft drinks in many countries (CDC, 2012; Currie *et al.*, 2008).

Regarding eating patterns of Italian children, the most recent *Okkio alla salute* findings show that 8% of children skip breakfast, 31% consume an inadequate breakfast (i.e. unbalanced carbohydrate/protein ratio), 52% consume a hyper-caloric morning snack, 25% of parents declare that their children do not eat fruit and vegetables every day and 41% of parents declare that their their children regularly consume soft drinks (Spinelli *et al.*, 2011).

In adolescents the most recent HBSC report (Currie *et al.*, 2012) showed that food habits worsen from 11 to 15 years old. Prevalence of daily breakfast consumption declined significantly in boys and girls from 70% to 55%; fruit and vegetable regular consumption decreased from 42% at 11 years old to 31% at 15 years old; soft drink consumption increased with age from an average of 18% of 11 year-old adolescents who consume soft drinks every day to an average of 25% at 15 years old (boys consume more soft drinks than girls).

Regarding calories expenditure, different indicators were used by *Okkio alla Salute* to evaluate sedentary behaviour and physical activity. Forty two per cent of children have a TV in their bedroom; 35% watch TV and/or play videogames > 2 hours/day; 18% play sports for not more than 1 hour/week and 1 child out of 4 goes to school on foot or by bicycle.

HBSC reported a worsening attitude with age also for sedentary behaviour and physical activity; in fact, 23% of 11 year-old adolescents undertake at least 1 hour of Moderate-to-Vigorous Physical Activity (MVPA)/day vs. 19% of 13 year-olds and 15% of 15 year-olds; moreover, 56% of 11 year-old adolescents watch TV > 2 hours/day, while at 15 years old the percentage rises to 63%.

1.4 CONSEQUENCES OF AN UNHEALTHY LIFESTYLE DURING CHILDHOOD

The lifestyle of individuals is very vulnerable to negative influences during childhood and adolescence, which are crucial periods of life when both major physical and psychological changes occur. The risks associated with unhealthy behaviour related to diet and physical activity begin in childhood and build up throughout life (WHO, 2003; WHO, 2010a).

During adolescence, diet may affect health status also in the short term, for instance by increasing adiposity and negatively affecting cardiovascular risk factors (Davis *et al.*, 2007); similarly, sedentary behaviour and low physical activity are associated with excess body fat, hypertension and high cholesterol levels (Freedman *et al.*, 1999).

Obesity often persists into adulthood, increasing children's risk of developing cardiovascular disease, type 2 diabetes and other chronic diseases later in life (Lobstein *et al.*, 2004). For most NCDs resulting from obesity, the risks depend partly on the age of onset and on the duration of obesity. Obese children and adolescents suffer from both short-term and long-term health consequences.

There is also good evidence that obesity is associated with high intake of energy-dense, nutrient poor foods such as soft drinks, savory crisps and sweet biscuits, and increased time spent in sedentary behaviour (Carlson *et al.*, 2012, WHO, 2003). On the other hand, regular physical activity and a diet high in fruit, vegetables, legumes and whole-grain cereals have been shown to be protective (Swinburn *et al.*, 2004; WHO, 2003).

The most significant health consequences of childhood overweight and obesity, that often do not become apparent until adulthood, include:

- cardiovascular diseases (mainly heart disease and stroke);
- diabetes;
- musculoskeletal disorders, especially osteoarthritis;
- certain types of cancer (endometrial, breast and colon).

Moreover, many low- and middle-income countries are now facing a "double burden" of disease: on one hand they struggle with the problems of infectious diseases and under-nutrition, and on the other hand are experiencing a rapid increase in risk factors of NCDs such as obesity and overweight. This is particularly evident in urban settings. It is not uncommon to find undernutrition and obesity existing side-by-side within the same country, the same community and even within the same household in these settings (WHO, 2004).

This double burden is caused by inadequate pre-natal, infant and child nutrition which is then followed by exposure to high-fat, energy-dense, micronutrient-poor foods and a lack of physical activity as the child grows older.

1.5 NUTRITION AND LIFESTYLE FOR HEALTH: THE RECCOMENDATIONS

Healthy nutrition contributes to decreasing the risk of today's leading health problems in children and adolescents (including obesity, cardiovascular diseases, cancer and eating disorders) and strengthens the learning potential and well-being of children and adolescents. Currently there is no WHO dietary recommendation of global utility available for children and adolescents. However, individuals and populations are advised to:

- increase the consumption of fruit and vegetables, as well as legumes, whole grains and nuts;
- limit the energy intake from total fats and shift fat consumption away from saturated fats to unsaturated fats;
- limit the intake of sugars.

Child fruit consumption promotes optimal health, growth and intellectual development, lower levels of body fat and, in combination with vegetables, better bone density for boys (Vatamparast *et al.*, 2005). Eating fruit at younger ages appears to translate to adult patterns (te Velde *et al.*, 2007), with adult outcomes including decreased risks for coronary heart disease (Dauchet *et al.*, 2005), stroke (Dauchet *et al.*, 2006) and cancer (Maynard *et al.*, 2003). Most of the official and governmental documents recommend the consumption of 5 servings of fruit and vegetables/day both to adults and children (INRAN, 2003, USDA, 2010; WHO, 2004; WHO, 2010a).

One of the strategies for children and adolescents for limiting the energy intake from total fats and shifting fat consumption from saturated fats to unsaturated fats is avoiding or limiting snack consumption, which are very often high in saturated fats (Albers *et al.*, 2008).

The consumption of soft drinks has risen across the globe, accompanied by an increase in the prevalence of overweight and obesity. Regular consumption has been associated with increased energy intake, weight gain, risk of overweight and obesity, and the development of obesity-related chronic metabolic diseases such as metabolic syndrome and type 2 diabetes (Malik *et al.*, 2010). Moreover, soft drink consumption has been associated with lower intakes of milk, calcium and other nutrients (Vartanian *et al.*, 2007).

Regular breakfast consumption is associated with higher intakes of micronutrients, a better diet that includes fruit and vegetables and less frequent use of soft drinks (Timlin *et al.*, 2008). BMI and the prevalence of overweight are in general lower in young people who eat breakfast (Affenito, 2007), which is also advocated as a means of improving cognitive function and academic performance (Cooper *et al.*, 2011). Moreover, breakfast consumption is also associated to the consumption of milk and yoghurt, which is promoted by USDA (2010) and other national guidelines.

Regarding physical activity, this has been shown to be essential for longand short-term physical and mental health outcomes (Hallal et al., 2006; McMurray et al., 2008) and may improve academic and cognitive performance (Martines-Gomez et al., 2011; McMurray et al., 2008). Physical activity is also associated with increased musculoskeletal and cardiovascular health and reduced anxiety and depression among young people (Strong et al., 2005). Good physical activity habits established in youth are likely to be carried through into adulthood (Iannotti et al., 2009), while lower physical activity levels and excess sedentary behaviour are associated with obesity (Sibley and Etnier, 2003). Based on their extensive review of the literature, Strong et al. (2005) recommended that children participate in at least 60 minutes of MVPA daily (MVPA has been defined as "any activity that increases your heart rate and makes you get out of breath some of the time"), offering country-specific examples of such activities. This minimum standard has been included in guidelines issued by some governments and professional organizations, but evidence suggests that a significant proportion of young people do not meet it (Borraccino et al., 2009).

It has to be underlined that for children and young people, physical activity includes play, games, sports, transportation, recreation, physical education, or planned exercise, in the context of family, school, and community activities. These activities are fun for children and adolescents, therefore they can be continued overtime and are aerobic, as they should be. Moreover, to meeting the goal of 60 minutes per day it can be suggested also to perform activities in multiple shorter bouts spread throughout the day (e.g. 2 sessions of 30 minutes), then adding together the time spent during each session.

Physical activity has also been associated with psychological benefits in young people by improving their control over symptoms of anxiety and depression. Similarly, participation in physical activity can assist in the social development of young people by providing opportunities for self-expression, building self-confidence, social interaction and integration. It has also been suggested that physically active young people more readily adopt other healthy behaviour (e.g. avoidance of tobacco, alcohol and drug use) and demonstrate higher academic performance at school (Currie *et al.*, 2012).

The last, but not less important, recommendation is to reduce sedentary behaviour. Sedentary behaviour refers to an absence of or minimal involvement in physical activity, and low energy expenditure (Biddle *et al.*, 2004). Although HBSC analyses show weak or no relationship with reduced physical activity (Borraccino *et al.*, 2009; Janssen *et al.*, 2005), sedentary behaviour is a cardiovascular-disease risk factor independent of low physical-activity levels (Hume *et al.*, 2009). In addition, screen-based sedentary behaviour have been related to other adverse health behaviour and negative health indices, such as substance use, health complaints and aggression (Iannotti *et al.*, 2009). Its effects are cumulative over the course of childhood, with television viewing during adolescence being associated with weight gain in adulthood (Parsons *et al.*, 2008). In fact, interventions

targeting sedentary behaviour in children result in weight reduction (DeMattia *et al.*, 2007). Current recommendations suggest that children should have no more than 1–2 hours of high-quality television and/or screen time per day, but most exceed these limits (Canadian Paediatric Society, 2003).

For inactive children and youth, a progressive increase in activity to eventually achieve the target shown above is recommended. It is appropriate to start with smaller amounts of physical activity and gradually increase duration, frequency and intensity over time. It should also be noted that if children are currently doing no physical activity, doing small amounts, even if much below the recommended levels, will bring more benefits than doing none at all.

1.6 WHAT IS THE MEDITERRANEAN DIET?

"What is Mediterranean Diet? One definition might be that it is what the Mediterranean natives eat. But as we know and think of it now, it is a relatively new invention. Tomatoes, potatoes, and beans, for example, came from America long after Christopher Columbus discovered the New World.

The heart of what we now consider the Mediterranean Diet is mainly vegetarian, pasta in many forms, leaves sprinkled with olive oil, all kinds of vegetables in season, and often cheese, all finished off with fruit, and frequently washed down with wine"

Ancel Keys, 1995.

The provocative definition of the most important scientist in the discovery of the correlation between the diet and health highlights the ambiguity of the term "Mediterranean Diet" and the difficulty in defining it. He suggests that it can be what the Mediterranean natives ate.

The term "Mediterranean Diet" has been widely used to describe the traditional dietary habits of people living around the Mediterranean basin, in particular in the olive tree growing areas, whereas "traditional" means before the globalization expanded also to the food culture.

Mediterranean Diet (MD) is characterized by abundant plant foods (fruit, mainly as typical daily desserts, vegetables, bread, other forms of cereals, beans, nuts and seeds). It also includes olive oil as the principal source of fat, moderate amounts of dairy products (principally cheese and yoghurt), low to moderate amounts of fish, low amounts of poultry and red meat and red wine consumed in low to moderate quantities, normally with meals (Kafatos *et al.*, 2000; Willet *et al.*, 1995).

The Mediterranean dietary pattern is characterized as rich in monounsaturated fatty acids, a balanced ratio of (n-6):(n-3) essential fatty acids, and high amounts of fibre and antioxidants, such as vitamins E and C, resveratrol, polyphenols, selenium, and glutathione (Simopoulos, 2001). Moreover, seasonality, biodiversity, the use of traditional and local food products are also important elements in this pattern.

MD has also qualitative cultural and lifestyle elements, such as frugality, sobriety, conviviality, culinary activities, physical activity and adequate rest (Bach-Faig *et al.*, 2011).

Many different communities live around the Mediterranean Basin, encompassing different religions, different cultures, different economic systems and therefore different traditions and diets. In this sense, we can consider not just a single MD but many Mediterranean Diets. Religion influences dietary habits, for example Muslims do not eat pork or drink wine and other alcoholic drinks, whereas the Greek Orthodox usually do not eat meat on Wednesdays and Fridays but drink wine.

Three continents and 22 countries border the Mediterranean sea, and despite their different religions, cultures and ethnic groups, they have some common tracts in their food habits: the consumption of olive oil, bread, vegetables and legumes, fruit and nuts, and also the taste for conviviality. And, of course, the use of traditional and local ingredients help to maintain biodiversity (Local Food-Nutraceuticals Consortium, 2005).

Historical antecedents

Knowledge of the ancient MD (around the time of the Greeks and Romans) comes from a vast archeologic record of food debris, a food-related art, pottery, literature and inscribed tablets that have been excavated in the Mediterranean region from various archeological sites (Fidanza, 1979). Despite the difficulty in evaluating such evidence, scholars have documented the availability of an astonishing variety of plant and animal foods, spices, bread, sweets, beer and wine (Darby *et al.*, 1977; Seymour, 1907; Vermeule, 1964; Vickery, 1936). The presence of these foods in a region suggests but not proves that they were regularly consumed. There are many sources, especially by Classical authors that document only the habits of noblemen or warriors, who probably ate almost only meat, bread and wine (Seymour, 1907). Homeric texts mention vegetables and fruits only rarely, perhaps because such foods were "considered beneath the dignity of gods and heroes" (Yonge, 1909). Moreover, olive oil was cited only as an unguent (Seymour, 1907). Modern scholars, though, have concluded that the typical diet of the

common people must have been rather sparse and based mainly on plants foods and bread, with meat and seafood only for special occasions (Seymour, 1907; Vickery, 1936).

The Rockfeller Foundation's Study

The first systematic attempt to investigate dietary intake in the Mediterranean region took place just after the World war II. In 1948, Greece government invited the Rockefeller Foundation to undertake a large and complex epidemiological study on the island of Crete with the aim of understanding how to raise the population's standard of living. The survey included: 7 days weighted food inventories collected from 128 households; 7 days dietary intake records of more than 500 subjects; food frequency questionnaires administered to 765 households; a review of food balance data for Greece; questionnaires on cooking practices, daily menus and food expenditure for 128 households; 2 days dietary intake surveys of pregnant and lactating women; 2 days dietary intake surveys for children 7-19 years old; and a survey of the dietary intake of children 1-6 years old obtained from their parents (Allbaugh, 1953).

The results of the Rockfeller Foundation's study are well summarized in Table 1.1 and Table 1.2. Allbaugh's conclusions were that "olives, cereals, grains, pulses, wild greens and herbs, and fruits, together with limited quantities of goat, meat and milk, game and fish remained the basic Cretan foods for forty centuries [...] no meal was complete without bread [...] Olives and olive oil contributed heavily to the energy intake [...] food seemed literally to be "swimming" in oil" (Allbaugh, p.100). Moreover, wine was consumed frequently, at all meals.

Allbaugh found that Cretan diets were generally nutritionally adequate and that the food consumption levels observed in most individuals were "surprisingly good. On the whole, their food patterns and food habits were extremely well adapted to their natural and economic resources as well as their needs" (Allbaugh, p.31).

This view was not shared with the inhabitants of the Cretan island. Allbaugh reported that only one of six of the interviewed households judged their daily consumed diet as satisfactory. He quoted one family stating: "We are hungry most of the time" (Allbaugh, p.105). Survey respondents listed, in priority order, the followings as most desired foods: meat, rice, fish, pasta, butter and cheese. Meat was very frequently the favorite food. Among Allbaugh's conclusions there was the suggestion of improving the Cretan diet with foods of animal origin (Nestle, 1995).

The Seven Countries Study

MD drew the attention of scientists during the early post World War II period, mostly because a link between the dietary habits of Mediterranean populations and health indicators was unexpectedly discovered. The largely ecological, yet insightful, research by the legendary Ancel Keys and his colleagues (Keys *et al.*, 1980) brought the concept of MD into the mainstream of science focusing on the relation between nutrition and health (Trichopoulou, 2012).

Keys went to South Italy (Naples province) in the '50s observing a low prevalence of coronary heart disease among the people of a low sociocultural level. During the famous "Seven Countries Study", started in 1957, Keys and his colleagues discovered that the less wealthy people had different food habits from the rich: they ate much less meat and animal fat, a large amount of vegetables, legumes and pasta. Moreover, they ate fruit as dessert, they were active people and had adequate rest. Italian diets were remarkably low in fat (20% of energy), just half the proportion observed in the diets of comparable American groups (Keys *et al.*, 1954). By that time, long before such ideas became commonplace, Keys had associated the typical American diet, rich in meat and dairy fats, with high concentrations of blood cholesterol and, therefore, with increased risk of coronary heart disease (Nestle, 1995). Keys was also very touched by the existence of small and family restaurants (*trattorie*), which did not exist in the United States and by the socio-cultural meaning of conviviality (Lucchin, 2012).

Keys liked to describe the dietary habits he and his wife found in Southern Italy as a diet including:

"homemade minestrone [...] pasta in endless variety [...] served with tomato sauce and a sprinkle of chees, only occasionally enriched with some bits of meat, or served with a little local sea food [...] a hearty dish of beans short lengths of macaroni [...] lots of bread never more than a few hours from the oven and never served with any kind of spread; great quantities of fresh vegetables; a modest portion of meat or fish perhaps twice a week, wine of the type we call *Dago red* [...] always fresh fruit for dessert. Years later, when called on to devise diets for the possible prevention of coronary heart disease we looked back and concluded it would be hard to do better than imitate the diet of the common folk of Naples in the early 1950s" (Keys A. and Keys M., 1975, p.4).

The Seven Countries Study was an extensive and important multi-centric cross-sectional study: 12,763 40-59 years old subjects were recruited from 7 different countries (Finland, Japan, Greece, Italy, the Netherlands, the United States and former Jugoslavia) and tracked for 40 years. In Italy the sample was recruited in Nicotera in the Calabria region, Crevalcore in the Emilia region, Montegiorgio in the region of Marche, Pioppi, in the Cilento area, in the Campania region (Keys *et al.*, 1980). Keys was so impressed by the beauty of Cilento, the excellence and wholesomeness of the food that he spent there 28 years with his wife.

The results of the Seven Countries Study were incontrovertible (Figure 1.2): the population of the Mediterranean Basin who consumed a diet mainly of pasta, fruit and vegetables, moderate quantities of fish and olive oil as the main condiment had a much lower percentage of mortality from ischemic heart disease than populations, such as the Finnish, who included in their diet many saturated fats of animal origin, such as in butter, milk, meat and cheese (Menotti *et al.*, 2007).

The EURATOM Study

From 1963 to 1965 the European Atomic Energy Commission (EURATOM) examined household food consumption in 3,725 families in 11 regions of 6 European countries in order to identify commonly consumed foods that were mostly likely to be sources of radioactive contaminants (Cresta *et al.*, 1969). There were conducted dietary interviews on 7 consecutive days and weighted all foods present in the household in those days. After applying several correction factors, the investigators converted the data on household food consumption to average amounts of food consumed per person per day.

The EURATOM study provided comparative information about dietary intake in the Mediterranean and other regions of Europe. Nine of the regions selected for the study were in Northern Europe and two in Southern Europe. Because one of the Northern European sample was Friuli, in Northern Italy and both of Southern European samples were in Southern Italy (Campania and Basilicata) the data can be used to compare the typical dietary intake of Northern Italy inhabitants with those of Mediterranean regions ones (Ferro-Luzzi and Branca, 1995).

The EURATOM study describes many differences in dietary intake patterns among the Italian regions. Diets of the Mediterranean areas were characterized by a larger amounts of cereals, vegetables, fruits, and fish, but a much smaller intake of potatoes, meat and dairy foods, eggs and sweets. The quantity of fat was not different among the two different populations, but the quality did. Consumption of butter and margarine was much higher in Northern regions, while in the South the principal source of fat was olive oil and margarine was not consumed at all.

EURATOM study is a further evidence that the Mediterranean Diet of the mid-1960s was based mainly on plant foods and included olive oil as the principal fat.

1.7 MEDITERRANEAN DIET AND HEALTH

The first evidence of the possible beneficial effects on health, and in particular on the cardiovascular diseases (CVDs) of the MD is the results of Keys' study (Keys *et al.*, 1986). The low incidence of CVDs in the populations of the Mediterranean basin in the Seven Countries Study opened a new research field with the aim of confirming these results.

The most important recent epidemiological data are the ones derived from the Elderly Prospective Cohort Study (EPIC), multicentric prospective study that analysed subjects from 10 different countries (Thricopoulou *et al.*, 2003). Starting from the conclusions of this study it has been possible to highlight how the adherence to the MD is able to lowering the incidence of CVDs. Thricopoulou *et al.* (1995) have established a score for assessing the adherence to MD, which takes into account the main food groups typical of the MD (Mediterranean Diet Score - MDS). A subject received a point if his intake was over the sample median for a protective component (vegetables, fruits, etc.) and below the median for non-protective components (dairy products, meat, etc.). A score equal to 0 (zero) indicates, therefore, a low level of adherence to the MD, while a score of 8 represents the maximum adherence to the MD (see chapter 1.8 for the description of all indexes). Through the adoption of this score it was possible to document that the mortality risk was inversely correlated to the level of adherence to the MD in subjects followed for 4 years. A 2 point increase in MDS was significantly associated with a reduction of 33% of mortality for CVDs, after adjustment for the confounding factors. These data have been also confirmed by more recent Spanish studies (Martinez-Gonzales *et al.*, 2011).

The protective effect of the MD on the CVDs has been shown also in countries far from the Mediterranean basin, as the U.S.A.. Mitrou et al., (2007) analysed 214,284 subjects from 50 to 71 years of age for a mean follow up period of 10 years. The subjects with a higher level of adherence to the MD showed a reduction of 32% of cardiovascular mortality in both sexes. Moreover, Sofi et al. (2008 and 2010) published 2 interesting meta-analysis on the epidemiological prospective studies which evaluated the adherence to the MD through a quantifying score and the related health status. The metaanalysis presented the results of 12 population studies, which globally had studied 1.5 million people in a follow up period that lasted from 3 to 18 years. Clinical events included in this analysis were: mortality for all causes, incidence of CVDs, incidence of cancers and incidence of neurodegenerative diseases as Parkinson's and Alzheimer's. From the global analysis of all selected studies it is possible to highlight that an increase of 2 points in the level of adherence determined a reduction of 9% of the incidence of CVDs.

The correlation between eating patterns and insurgence of cancer has been shown in several studies. Various aspects of the MD resulted positive for the reduction of the incidence of different types of cancer. It has been suggested how the adoption to a Mediterranean eating pattern can prevent about 25% of colon cancer, 15-20% of breast cancer and 10-15% of prostate and pancreas cancer (Trichopoulou *et al.*, 2000). These data have been confirmed by the EPIC study which followed 22,000 people for 4 years and found that a high adherence to the MD determined a reduction of 24% of deaths from cancers (Trichopoulou *et al.*, 2003). Similar results have been obtained in the large sample of 350,000 subjects from U.S.A., followed for more than 5 years (Mitrou *et al.*, 2007). Finally, Sofi's meta-analysis revealed that 2 points increase in MDS reduces the incidence and/or the mortality from cancer by 6% (Sofi *et al.*, 2008).

Recent evidence show how the adherence to the MD have an important role also on the neuro-degenerative diseases, as Parkinson's and Alzheimer's. The first interesting study which hypothesized the relation between MD and neuro-degenerative diseases was published in 2006 (Scarmeas *et al.*, 2006). The authors studied 2258 subjects, evaluated with a test for cerebral function every 1,5 years for 4 years. At the end of the observational period 262 cases of Alzheimer disease were observed. In this sample a better adherence to the MD was found to decrease the risk of insurgence of the disease. In the same way, the association between adherence to the MD and risk of insurgence of Parkinson's disease has been found in a population of 49,692 men and 81,676 women recruited for the Health Professionals Follow-up Study (Gao *et al.*, 2007). The subjects who were in the highest quintile of adherence to the MD showed a decrease of 25% of the risk of insurgence of Parkinson's disease in comparison with the subjects in the lowest quintile of adherence.

The MD is a useful and valid not-pharmacological help in the therapy of type-2 diabetes. This evidence is very well shown in the results of the study by Martinez-Gonzales *et al.* (2008) who studied the incidence of diabetes in a cohort of 13,380 subjects with a mean age of 37 years old, followed for 4 years. The results, adjusted for age and sex, highlight that the subjects with a

high adherence to the MD (MDS>6) had a reduction of the risk of diabetes' insurgence of 83% in comparison with the subjects with a lower adherence.

Adhere to the MD not only do not determine a weight increase in normal weight people, but also protect from the risk of overweight and obesity. The EPIC study is very helpful to confirm this thesis: a sub-cohort of 497,308 subjects (25-70 years old), from 10 European countries was analysed with the objective of evaluating the possible relationship between adherence to the MD and BMI. The results of the study highlight that, in a 3 years period, a high level of adherence is significantly associated with a BMI and WC reduction (Romaguera *et al.*, 2009).

Finally, in children and adolescents, adherence to the MD is associated both at long and short term with health indicators. We have observed that a healthy child/adolescent has more probability to become a healthy adult and that the MD is significantly associated with health in adulthood. MD adherence can also contribute to the health of children and adolescents in the short term, as del Mar Bibiloni *et al.* (2011) show in their study on metabolic syndrome in adolescents from the Balearic islands. The results of this study show how higher adherence to the MD was associated with significantly lower odd ratio of having metabolic syndrome.

1.8 METHODS FOR THE EVALUATION OF THE ADHERENCE TO THE MEDITERRANEAN DIET IN ADULTS

To analyse food patterns there are two approximations: developing food indexes, i.e. food scores according to the intake of certain foods; or deriving patterns via multi-variant analysis by means of a factorial analysis, principal component analysis or cluster analysis (Hu, 2002).

Adherence to the MD has been evaluated through various scores which holistically reflect the dietary exposure when evaluating diet disease associations (Naska and Trichopoulou, 2014).

The MD indexes attempt to make a global evaluation of the quality of the diet based on a traditional Mediterranean 'reference' pattern, described as 'a priori', being general and qualitative. The Mediterranean diet indexes, hence, summarise the diet by means of a single score that results from a function of different components, such as food, food groups or a combination of foods and nutrients. These components are previously selected based on prior knowledge or scientific evidence, this approach thus being an 'a priori approximation' (Bach *et al.*, 2006).

A recent review on food indexes highlighted that the various indexes differ for many aspects (for example the components used to develop the score and the cut offs), indicating that the choices to develop the indexes are arbitrary (Waijers *et al.*, 2007). The existent food indexes obtained to measure dietetic quality and the adherence to the MD, are probably predictors of mortality significantly more than the single dietetic factors.

The indexes vary for the following aspects: number of components, cut offs for the category classification, statistical parameters (mean, median, tertiles, quintiles) and contribution of the single components (positive, negative) to the total score (Bach *et al.*, 2006).

The methods to evaluate the adherence to the MD can be classified in 3 categories depending on the way they are calculated: 1) those based on a positive or negative scoring of the components; 2) those that add or substract

standardized components, and 3) those that are based on a ratio of components (Bach *et al.*, 2006).

Irrespective of how conformity to the Mediterranean diet is computed, all scores share some common ground; they are all based on the above dietary components that capture the essence of this dietary profile.

1.8.1 Indexes by positive or negative scoring of the components

Mediterranean Diet Score

The first and more often cited index to assess adherence to the MD among adults is the Mediterranean Diet Score (MDS) created by Trichopoulou *et al.*, (1995), illustrated in Table 1.3.

The Traditional Greek MD was simplified into eight components to define the MDS (Trichopoulou et al., 1995): (1)high ratio of monounsaturated:saturated fat (MUFA:SFA), (2) moderate alcohol intake, (3) high legume intake, (4) high intake of grains (including bread and potatoes), (5) high fruit intake, (6) high vegetable intake, (7) low intake of meat and meat products and (8) moderate intake of milk and dairy products. The MDS was based on assigning a score from 0 to 1 according to the daily intake of the eight components. In general, the medians of the sample, specific for sex, were used as cut-off points (and grams per day were used as the intake measurement (Trichopoulou et al., 1995). A subject received 1 point if his intake was over the sample median for a protective component (vegetables, fruit, etc.) and below the median for non-protective components (dairy products, meat, etc.). In the case of alcohol (except when specified) 1 point was scored for males if their consumption was within 10 and 50 g/day, and within 5 and 25 g/day for women. If all the characteristics of the diet were incorporated, the highest score was obtained and reflected a greater

adherence to the MD. Therefore, the MDS usually ranged from 0 (minimal adherence) to 8 (maximum adherence) if the index had eight components. Generally, a score of 4 or more was associated with satisfactory MDP adherence and better health implications (Osler and Schrool, 1997; Trichopoulou *et al.*, 1995). In most studies, intake was adjusted for calories consumed, 2500 kcal for men and 2000 kcal for women, so the estimations would be independent of the variations present in energy intake.

Eight years later Trichopoulou *et al.* (2003) published a study with a variant of the first MDS, differing from the initial 8 points one. The new version incorporated fish and moderate poultry intake. In 2004, Psaltopoulou *et al.* (2004) proposed another version with 9 components. Moreover, different variants of the original index were elaborated for different non-Mediterranean populations, as Danish (Osler and Schrool, 1997), Australian (Kouris-Blazos *et al.*, 1999) and Chinese (Woo *et al.*, 2001).

Operational Mediterranean dietary pattern index

To understand if the MD plays a protective role in cardiovascular death, a 'post hoc' index was created based on 'a priori' defined MD pattern (Martinez-Gonzales *et al.*, 2002). The 'a priori' index was made up of eight components, where apart from foods, such as those with high glycaemic index, nutrients could also be found. According to the quintile distribution by the intake of each component (adjusted by energy) a score was assigned from 1 to 5 for each element. In the case of protective components 1 point was assigned to the lower quintile and 5 points to the upper quintile, and for the non-protective components, the scoring was inverted. In this way, each participant had a total score that ranged from 8 to 40. In the 'post hoc' index a single cut-off point was used for each component based on the 'previous' results of the dose–response associations between the intake of each component and the risk of suffering a second myocardial infarction. For the

majority of index components, individuals in the second quintile had a major reduction of risk in comparison to those in the first quintile, but no significant differences between Q2 and Q5 were observed. Therefore, with the 'post hoc' index if the participant consumed more than this value (Q2) 1 point was assigned for each component, thus obtaining a score between 0 and 8. The results of both indexes indicated that when the score increased, the odds ratio for myocardial infarctions was significantly reduced.

Two indexes were created on the basis of the Mediterranean food pyramid (Bach *et al.* 2006): a) *Meditterranean score* by Goulet *et al.* (2003); b) *Dietary score* by Panagiotakos *et al.* (2006).

Meditterranean score

The *Meditterranean score* was based on a study on Canadian women and food habits, plasma lipoprotein profiles and body weight modifications in a 12-week nutritional intervention promoting the MD (Goulet *et al.*, 2003). Scoring based on 11 components of the Mediterranean pyramid by Oldways Preservation Trust was designed to evaluate MD adherence. A partial score of 0 to 4 was attributed to each component. Food found at the base of the pyramid received a high score when consumed frequently. However, food found at the peak of the pyramid (meat, sweets and eggs) was given a high score when consumed less frequently. From the nutritional intervention, it was observed that the 'Mediterranean score' sample mean increased significantly from 21.1 points to 28 points, and resulted in a slight but significant improvement of the metabolic profile (total cholesterol, apo-B levels and BMI), a significant decrease in energy coming from lipids and a significant reduction in weight and waist circumference.

Dietary score

The *Dietary score* was also constructed on the Mediterranean Diet Pyramid to study the differences in plasma lipids according to MDP adherence (Panagiotakos *et al.*, 2006). The index was constructed based on higher scoring from 0 to 5 points according to intake frequency of typical Mediterranean products, and for those components from the MD a decreased scoring. The adherence to the MD resulted in significant reductions only for levels of oxidised LDL-cholesterol.

Mediterranean Diet Quality Index

The Mediterranean Diet Quality Index (Med-DQI) was an adaptation of the "Diet Quality Index" (Patterson *et al.*, 1994) to evaluate the MD adherence. Olive oil, fish, and alternative meat substitutes were added. The objective of the study was to evaluate the adherence to the MD in a French population, to study the socio demographic and lifestyle associated factors and to validate and correlate the Med-DQI with biological markers (carotene, vitamin E, EPA and DHA). In the Med-DQI a score from 0 to 2 was assigned to each food group according to the recommendations when existing, or otherwise using the population intake tertiles to assign cut-off points. The total score of the index was from 0 to 14 points. The lower the Med-DQI value, the healthier the diet. Elder individuals and those living in rural areas, working class individuals and non-smokers presented a greater Med-DQI. There was a high correlation between Med-DQI and studied biomarkers, with the exception of cholesterol.

Mediterranean Diet pattern score

In an Italian cohort study, a score was created to be able to study the association between the MD adherence and the risk of developing peripheral vascular disease in type -2 diabetic patients (Ciccarone *et al.*, 2003), as it was postulated that more than a specific food alone, a high score may play a protective role. A score was developed where a point was added according to food intake with sufficient evidence of its beneficial effect on coronary artery disease, and 0 for those foods that have potentially harmful effects.

Specifically, the study found that individuals who obtained 11 points or more had 56% less risk of suffering this illness.

1.8.2 indexes that adds and subtracts standardised components

The SUN study is a prospective cohort study based on a sample of 4259 University of Navarra students with the objective of assessing the protective role of the MD on coronary diseases and evaluating variables associated with this diet (Sanchez-Villegas *et al.*, 2002). An index was built defining MD as "a priori" by summing the standardized residuals of nutrients and foods after adjusting a regression model using total energy intake as the independent variable. The adjusted intake of each food group was standardised as z-value (observed mean/observed standard deviation). The results suggested that there is a progressive abandonment of the traditional MD in younger individuals and those individuals who led an active lifestyle had greater adherence to the MD.

In a transverse study of similar characteristics carried out in the Balearic Islands (Tur *et al.*, 2004) a variant of the Sanchez-Villegas index was applied. It also had the objective of analysing the prevalence of the MD in a sample of 1,200 individuals and the socio-demographic and lifestyle factors related to this pattern. The adherence to the MD was defined through 9 points or characteristics, with small component modifications as compared to Sanchez-Villegas *et al.* (2002), but with the same calculation methodology. The MD adherence in the Balearic population was 43.1%, similar for all socio-demographic groups and lifestyles but with differences in age, sex, physical activity and smoking habits.

1.8.3 Indexes based on the ratio of components

Mediterranean Adequacy Index

The adherence to the Italian MD reference was measured in two Italian cohorts of the Seven Countries Study (Crevalcore and Montegiorgio) (Alberti-Fidanza *et al.*, 1999). The 'Mediterranean Adequacy Index' (MAI) was based on a quotient between the sum of energy proceeding from Mediterranean products (carbohydrate and protective food groups) and the sum of energy from non-Mediterranean products (animal origin foods and sweets). High index values indicated a greater MD adherence. During the 31-year follow-up, a progressive abandonment of the MD was observed, taking as a reference an Italian MD from the town of Nicotera. The MAI was also applied to Spanish Family Food Balance Sheets from the Ministry of Food and Agriculture (Fuentes-Bol, 2002).

1.9 METHODS FOR THE EVALUATION OF THE ADHERENCE TO THE MEDITERRANEAN DIET IN CHILDREN AND ADOLESCENTS

Two indexes were specifically created to assess the adherence to the MD in children and adolescents: the Mediterranean Diet Quality Index for Children and Adolescents (KIDMED) by Serra-Majem *et al.* (2004) and the food frequency-based Mediterranean Diet Score (fMDS) by Tognon *et al.*, (2014a). Moreover, some authors used various modifications of the MDS adapted for children and adolescents (see Chapter 1.9.3). The most used index is, however, the KIDMED index.

1.9.1 Mediterranean Diet Quality Index for children and adolescents (KIDMED)

The KIDMED was developed by Serra-Majem *et al.* (2004) in a sample of 3,850 children and youths aged 2–24 years, drawn from the EnKid Study population (in which two 24-hour recalls, a quantitative 169-item food-frequency questionnaire and a general questionnaire about socio-economic, demographic and lifestyle items were also administered).

The development of the KIDMED index was based on principles sustaining Mediterranean dietary patterns as well as those that undermine it. The index is based on a 16-questions test that could be self-administered or conducted by interview. Questions denoting a negative connotation for the MD have a value of - 1, and those with a positive aspect +1 (Table 1.4). The sums of the values from the administered test are classified into three levels: $(1) \ge 8 =$ optimal Mediterranean diet (category named "good); (2) 4-7 = improvement needed to adjust intake to Mediterranean pattern (category named "average"); $(3) \le 3$, very low diet quality (category named "poor").

1.9.2 Food frequency Mediterranean Diet Score (fMDS)

Adherence to a Mediterranean-like diet has been assessed by a food frequency-based Mediterranean Diet Score (fMDS) by Tognon *et al.*, (2014a). Daily frequencies from different food groups were divided by the total daily frequency of all food items included in a specific food frequency questionnaire to obtain relative frequencies of each food group. The latter values were categorized according to sex-specific and age-specific (6-9 years/2-5 years) medians (calculated on the whole cohort), to identify high and low intakes. One point was given for intakes higher than the median relative frequency for: (1) vegetables and legumes (cooked/raw vegetables, potatoes, legumes and vegetable-based meat replacements such as tofu,
tempeh, etc.), (2) fruit and nuts (fruit added or not with sugar, nuts, seeds, dried fruit), (3) cereals (breakfast cereals, white and whole meal bread, rolls and crispbread, pasta, noodles and rice) or (4) fish (fresh or frozen fish, and fried fish and fish fingers) and 1 point if intakes were below the median for: (5) dairy (milk, yoghurt, cheese including spreadable cheese) and (6) meat products (fried and non-fried meat, hamburgers, falafel, kebab, etc.). The final fMDS added up to a maximum of 6 points. High adherence levels to a Mediterranean-like dietary pattern were considered for subjects characterized by an fMDS > 3.

1.9.3 Adaptations of MDS for children and adolescents

Modification for children and adolescents are made by several authors.

Garcia-Marcos *et al.* (2007), based their score on Psaltopolous *et al.* (2004) and Trichopoulou *et al.* (2003) MDS. According to this index, fruit, fish, vegetables, legumes, cereals, pasta, rice, and potatoes are considered "pro-Mediterranean" foods and rated according the frequency of intake (0 points = never or occasionally; 1 point = 1 or 2 times/week; 2 points \geq 3 times/week). Meat, milk, and fast food are considered "anti-Mediterranean" foods and rated inversely (0 points \geq 3 times/week; 1 point = 1 or 2 times/week; 2 points = never or occasionally). Candies, industrial pastry, precooked pizzas and fried food, along with hamburgers taken in fast food restaurants, are classified generically as fast food.

Chatzi *et al.* (2007) and Jennings *et al.* (2011) utilized Trichopoulou's MDS (2003), presuming that dairy products are protective and not detrimental, and not including alcohol consumption in the index.

De Battle *et al.* (2008) modified Trichopoulou MDS (2003), suppressing the alcohol consumption component, and including a junk food and a fat consumption component, obtaining the usual range from 0 to 8.

1.10 AIMS AND OUTLINE OF THE THESIS

Unhealthy eating patterns and sedentary behaviour are linked with the onset of overweight and obesity and overweight obese children will probably become obese adults, with a shorter life expectancy and higher risk of contracting cardio-vascular diseases and cancer.

Moreover, childhood and adolescence are crucial periods of life when taste, food habits and lifestyle attitudes become established. This means that if young individuals learn to eat in a healthy way, and to be physically active, they will probably preserve these habits and their health in adulthood.

This work has been done with the aim of better understanding the factors associated with the high prevalence of overweight and obesity in the Campania region, the Italian region with the highest prevalence of childhood overweight and obesity, in order to support the urgent childhood obesity prevention programmes.

The specific aims are:

- to assess the association of eating and lifestyle patterns with different possible predictive factors such as age, gender and socio-economic status;
- to assess the association of eating and lifestyle patterns with adiposity indexes;
- to systematically review adherence to the MD in children and adolescents;
- to assess the association between adherence to the MD and possible predictive factors such as age, gender and socio-economic status;
- to assess the association between adherence to the MD and adiposity indexes.

The first study of the thesis is a cross-sectional survey on the clustering of eating and lifestyle behaviour in 478 adolescents (15-17 years old) from the Campania region. The aim was to assess the prevalence of meeting the international recommendations and the association between the cluster of recommended behaviour and abdominal adiposity.

The second study is a systematic review which aimed to analyse the level of adherence to the MD in children and adolescents and its possible predictive factors, its association with adiposity indexes, lifestyle and body composition variables and its nutritional adequacy.

The third study is a cross-sectional survey on eating patterns, lifestyle attitudes and the adherence to the Mediterranean Diet in 389 adolescents (11-13 years old) in the Campania region. The aim was to assess the prevalence of healthy and unhealthy eating patterns, lifestyle attitudes and level of adherence to the Mediterranean Diet, though the KIDMED index, and their associations with predictive factors and adiposity indexes.

The results of the three studies confirm the urgent need for prevention strategies and suggest possible tools for the implemention of effective strategies targeting specific populations.

Figure 1.1: Prevalence of overweight and obesity in Italian children: results from *Okkio alla salute*, 2014 (Nardone *et al.*, 2015).



Figure 1.2



MORTALITY BY CORONARY DISEASE IN THE STUDY OF SEVEN COUNTRIES AFTER 25 YEARS OF OBSERVATION

	Greece Food balance 1948-1949	Crete 7-d diet records	Household inventory ²
Energy (kcal/day)	2445	2547	2554
Foods (kg/person×year)			
Cereals	158.2	127.7	128.2
Potatoes	309	59.1	38.6
Sugar and honey	9.1	5.5	5.5
Pulses and nuts	15.0	20.0	23.2
Vegetables, fruits and olives	120.5	175.9	132.3
Meat, fish and eggs	23.2	28.6	27.7
Milk and cheese	35.0	25.5	34.5
Oils and fats	15.0	30.9	30.9
Wine, beer and spirits	37.7	10.0	38.6

Table 1.1. Results of the Rockfeller Foundation study: Dietary intake in Crete in 1948 by three methods¹

¹ Adapted from Allbaugh, 1953. ² Adjusted for information obtained from food-frequency questionnaires.

Table 1.2. Results of the Rockfeller Foundation study: Total energy and percent of total energy contributed by major food groups in Cretan diet compared with their availability in the food supplies of Greece and the United States in 1948-1949¹.

	Crete Greece 7-d record food balance		US food balance	
Energy (kcal/day)	2547	2477	3129	
Food group				
Cereals	39	61	25	
Pulses, nuts, potatoes	11	8	6	
Vegetables, fruits	11	5	6	
Meat, fish, eggs	4	3	19	
Dairy products	3	4	14	
Table oils and fats	29	15	15	
Sugar and honey	2	4	15	
Wine, beer, spirits	1	2		

¹ adapted from Allbaugh, 1953. ² Data not available

	Assigned point value							
	Never or occasionally	1 or 2 times per week	≥3 times per week					
Fruit	0	1	2					
Fish	0	1	2					
Vegetables	0	1	2					
Legumes	0	1	2					
Cereals	0	1	2					
Pasta	0	1	2					
Rice	0	1	2					
Potatoes	0	1	2					
Meat	2	1	0					
Milk	2	1	0					
Fast food ²	2	1	0					

 Table 1.3: Mediterranean Diet Score¹ (Trichopoulou *et al.*, 1995).

¹ After summarizing all of the points, higher scores mean greater adhesion to the Mediterranean Diet, and lower scores mean less adhesion.

²Candies, industrial pastry, precooked pizzas, and fried food, together with hamburgers taken in fast-food restaurants, were considered generically as "fast food."

Table 1.4. The KIDMED questionnaire and its scoring method (Serra-Majem*et al.*, 2004).

N.	Scoring	Question
1	+1	Takes a fruit or fruit juice every day
2	+1	Has a second fruit every day
3	+1	Has fresh or cooked vegetables regularly once a day
4	+1	Has fresh or cooked vegetables more than once a day
5	+1	Consumes fish regularly (at least 2–3 times per week)
6	-1	Goes more than once a week to a fast-food (hamburger) restaurant
7	+1	Likes pulses and eats them more than once a week
8	+1	Consumes pasta or rice almost every day (5 or more times per week)
9	+1	Has cereals or grains (bread, etc.) for breakfast
10	+1	Consumes nuts regularly (at least 2–3 times per week)
11	+1	Uses olive oil at home
12	-1	Skips breakfast
13	+1	Has a dairy product for breakfast (yoghurt, milk, etc.)
14	-1	Has commercially baked goods or pastries for breakfast
15	+1	Takes two yoghurts and/or some cheese (40 g) daily
16	-1	Takes sweets and candy several times every day

Score <4 = "poor" adherence Score 4-7 = "average" adherence Score > 7 = "good" adherence

CHAPTER 2: HEALTHY BEHAVIOUR AND ABDOMINAL ADIPOSITY IN ADOOLESCENTS FROM SOUTHERN ITALY

2.1 INTRODUCTION AND AIMS

The lifestyle of individuals is very vulnerable to negative influences during adolescence, which is a crucial period of life when both major physical and psychological changes occur. Overall, there is a worldwide trend to adopt unhealthy behaviour (Mazzarella and Pizzuti, 2011; Moreno *et al.*, 2010); for instance, both the Health Behaviour in School-aged Children (HBSC), a WHO collaborative cross-national survey in European countries (Currie *et al.*, 2008), and the Youth Risk Behaviour Surveillance System (YRBSS) in the USA (CDC, 2012) have indicated that skipping breakfast, low consumption of fruit and vegetables, high consumption of junk foods and increased sedentary behaviour are often observed in adolescents of both genders.

The risks associated with unhealthy behaviour related to diet and physical activity begin in childhood and build up throughout life (WHO, 2003; WHO, 2010b). During adolescence, diet may affect health status also in the short term, for instance by increasing adiposity and negatively affecting cardiovascular risk factors (Davis *et al.*, 2007); similarly, sedentary behaviour and low physical activity are associated with excess body fat, hypertension and high cholesterol levels (Freedman *et al.*, 1999).

Meeting health recommendations is expected to improve quality of life and to reduce the incidence of chronic diseases (Aarnio, 2003; Kavey *et al.*,

2003; WHO, 2004). Adolescents' behaviour can be modified through a number of different strategies, for example communication campaigns and educational counseling (Evans, 2009) following the most common national (INRAN, 2003) and international recommendations (USDA, 2010; WHO, 2004; WHO, 2010b) such as: (i) having breakfast; (ii) consumption of \geq 5 servings of fruit and vegetables/day; (iii) consumption of \geq 3 servings of milk/yoghurt daily; (iv) practice of Moderate-to-Vigorous Physical Activity (MVPA) for \geq 60 min/day; and (v) limiting watching television (TV) to <2h/day.

Notwithstanding, little is known in adolescents about either the clustering of dietary and physical activity behaviour or their effects on health outcomes (McKenna *et al.*, 1998; Sanchez *et al.*, 2007) and surrogate end points. Among the latter, waist circumference (WC), a widely recognized marker of abdominal fat, is of particular practical interest because it is a predictor of metabolic and cardiovascular risks in adults and children (Savva *et al.*, 2000; Takami *et al.*, 2001). As a matter of fact, WC and waist-to-height ratio (WHtR) are both able to identify children with a higher metabolic and cardiovascular risk better than BMI-for-age or skinfold thickness (Ashwell *et al.*, 2012; Freedman *et al.*, 2007; Saelens *et al.*, 2007). While WC has been shown to be related to each healthy behaviour (Alexander *et al.*, 2009; Bradlee *et al.*, 2010; Klein-Platat *et al.*, 2005; Martinez-Gomez *et al.*, 2010), no data are available regarding the association with clustered behaviour.

Moreover, the extremely high prevalence of childhood overweight and obesity has become a major public health issue in developed countries and world-wide (Waters *et al.*, 2011). Obesity often persists into adulthood, increasing children's risk of developing cardiovascular disease, type-2 diabetes and other chronic diseases later in life (Lobstein *et al.*, 2004). There is also good evidence that obesity is associated with high intake of energy-

dense, nutrient poor foods such as soft drinks, savory crisps and sweet biscuits, and increased time spent in sedentary behaviour (Carlson *et al.*, 2012, WHO, 2003).

On the other hand, regular physical activity (PA) and a diet high in fruit, vegetables, legumes and whole-grain cereals have been shown to be protective (Swinburn *et al.*, 2004; WHO, 2003).

Clustering or the co-existence of groups of people who share similar characteristics is a concept that has been successfully applied to understanding the relationships between different lifestyle behaviuor (Pronk *et al.*, 2004; Schuit *et al.*, 2002). The rationale underlying a focus towards clustering stems from the acknowledgement that the influences on lifestyle are multivariate and interactive (Pronk *et al.*, 2004). For example, diet, PA and sedentary behaviour may combine in complex ways that have a cumulative effect on the development of overweight and obesity (Sanchez *et al.*, 2007). This has important implications for public health because understanding which behaviour cluster together can be used to assist in the development of targeted obesity prevention initiatives. Interventions that are appropriately targeted and effectively bring about multiple behaviour change, may be more cost-effective and maximise reach, to those most in need (Prochaska, 2008; Prochaska *et al.*, 2008).

The major aims of the present study were to: (i) evaluate the prevalence of meeting recommendations for breakfast consumption, fruit and vegetable intake, milk/yoghurt consumption, MVPA levels and TV viewing in a sample of adolescents from southern Italy; and (ii) assess in these adolescents the association between meeting health recommendations and abdominal

adiposity. As a secondary end point, junk snack food consumption and its correlation with unhealthy behaviour were assessed.

2.2 METHODS

2.2.1 Study design and selection of participants

The study population was derived from a cluster-randomized sample of highschool students attending the second and third grade, participating in a study on the prevention of eating disorders (DiCAEv) in adolescence. That study took place in three high schools in Naples (Campania region, Italy). The sample included Caucasian individuals belonging to all socio-economic classes (according to parental educational level) and was representative of the school population of the Campania region (Mazzarella and Pizzuti, 2011). The adolescents' parents or guardians were fully informed about the objectives and methods of the study and signed a consent form. Also the adolescents provided their written assent. Study procedures were carried out according to the guidelines laid down in the Declaration of Helsinki and approved by academic and scholastic institution boards.

Inclusion criteria were: (i) age between 14 and 17 years and (ii) consent to participate. Six hundred and eighty seven adolescents were available to be recruited, but only 550 families gave their consent to participate in the study (80%); adolescents with missing data on any of the independent variables considered in the survey were excluded (n 72). Therefore a total of 478 adolescents were finally evaluated (participation rate 86.9%).

2.2.2 Anthropometric measurements

A portable scale (Seca model 813, Hamburg, Germany) was used to measure weight to the nearest 0.1 kg, with shoes and heavy clothing removed. Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca model 220). BMI (kg/m²) was calculated as body weight divided by the square of height. WC was measured at the narrowest point between the lower costal border and the iliac crest using a non-extensible steel tape (Seca model 200). Measures of height and waist were taken three times and the mean value was considered for data analysis. WHtR, a marker of abdominal fat deposition independent of age (Ashwell *et al*, 2012), was calculated and the cut-point of \geq 0.5 was used to identify adolescents with high abdominal adiposity (Ashwell and Hieh, 2005). Categories of overweight (OW) and obesity (OB) were defined according to the BMI thresholds proposed by Cole *et al.* (2000) for international comparisons, where the cut-off points for OW and OB are smooth sex-specific BMI centiles, constructed to match the values of 25.0 and 30.0kg/m², respectively, at age 18 years. Parental education level was considered as a proxy index of socio-economic status.

2.2.3 Dietary intake

In the present study, the consumption of breakfast (day/week) and that of four food items (fruit, vegetables, milk, yoghurt products) was explored. Usual dietary intake was assessed through a Food Frequency Questionnaire (FFQ) administered by interview by two trained nutritionists. For each food item, respondents were asked to indicate the frequency of consumption per day, per week or per month. Never and seldom were also included and considered as 'zero'. All reported numbers were converted to daily frequency (servings/day). The daily intake for each food group (fruit and vegetables; milk and yoghurt) was calculated by summing up the frequency of consumption of fruit plus vegetables or milk plus yoghurt. In the same way, the consumption (servings/day) of snack foods rich in fat and/or added sugars, such as crisps or chocolate bars - defined as 'junk snack foods' (Anderson and Patterson, 2005), was also assessed. Participants were classified as meeting each dietary recommendation when they consumed:

breakfast on ≥ 6 days/week, ≥ 5 servings of fruit and vegetables/d and ≥ 3 servings of milk/yoghurt daily (INRAN, 2003; USDA, 2010; WHO, 2004).

2.2.4 Physical activity

The modified long version of the International Physical Activity Questionnaire (IPAQ), as proposed by the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study (Craig et al, 2003; Hagströmer et al, 2008) and adapted into Italian according to the IPAQ committee guidelines (http://www.ipaq.ki.se/cultural.htm), was administered by trained operators. The questionnaire for adolescents focuses on four domains: (i) school-related physical activity (including activity during physical education classes and breaks); (ii) transportation; (iii) housework; and (iv) leisure time. The housework domain included only one question (compared with three in the original IPAQ) about physical activities in the garden or at home. For each of the four domains, the number of days per week and the number of physical activity periods per day (≥10 min of physical activity) were recorded. Outcome measures were average minutes of walking, moderate or vigorous activities per day; the sum of moderate and vigorous activities was computed to obtain minutes of MVPA per day. Participants were classified as meeting physical activity recommendations if they performed MVPA for ≥60 min/d (WHO, 2010b).

2.2.5 Sedentary behaviour

The study also included a questionnaire assessment regarding daily hours spent in TV viewing and computer or video game use. Average time spent watching TV (h/day) was used as a proxy for unhealthy sedentary behaviour because it is considered an important determinant of OW in adolescence compared with video game and computer use (Rey-Lopez *et al*, 2008). Participants were classified as meeting the specific recommendation if they limited TV viewing to <2h/day (WHO, 2010b).

2.2.6 Socio-economic status

Parental education level was considered as a proxy index of socio-economic status.

2.2.7 Statistical analysis

All statistical analyses were performed using the SPSS statistical software package version 18.0. The level of significance was set at p < 0.05. Variables were not normally distributed. Descriptive statistics, including medians, 25th and 75th percentiles, frequencies and percentages, were used to describe demographic and anthropometric characteristics of participants as well as healthy lifestyle behaviour. The non-parametric Mann–Whitney U test was used to compare differences in age, height, weight, BMI, WC and WHtR between genders. The Spearman's rank correlation test was performed to evaluate whether healthy lifestyle behaviour and junk snack food consumption correlated with each other. The χ^2 test was used to compare proportions for parental education levels or selected health behaviour between genders. The χ^2 test for trend was used for analysis of proportions between a binary variable (OW/OB status; WHtR ≥0.5) and an ordered categorical variable (number of risk factors). Finally, binary logistic regression analysis was performed to evaluate associations of each of the selected health recommendations with WHtR, controlling for age and gender.

2.3 RESULTS

2.3.1 Demographic and anthropometric characteristics

Demographic and anthropometric characteristics of the adolescents are shown in Table 2.1 and Table 2.2. No difference in BMI was found between genders, while WC and WHtR were higher in boys than girls. OW prevalence was higher in boys than girls (29.9% vs. 19.7%, respectively; p<0.01), while OB prevalence did not differ between genders (9.8% vs. 6.9%, respectively; p=0.2). WHtR was \geq 0.5 in 23.8% of boys and 10.3% of girls (p<0.01).

2.3.2 Meeting health recommendations

As median (25th–75th percentile) values, adolescents had breakfast on 7 (1– 7) days/week, consumed 1.6 (1.0-2.7) servings of fruit and vegetables/day, consumed 1.0 (0.3–1.0) servings of milk/yoghurt daily, reported 22.9 (11.1– 45.7) minutes of MVPA/day and spent 2.0 (1.0-2.5) h watching TV/day. The proportion of adolescents meeting each of the selected health recommendations is shown in Table 2.3; 55.4% for having breakfast, 2.9% for eating fruit and vegetables, 1.9% for milk/yoghurt consumption 13.6% for performing MVPA and 46.3% for TV viewing. No differences between genders were found, except that having breakfast and milk/yoghurt consumption that were slightly more frequent in boys. More than 65% of adolescents ate ≥ 1 serving of junk snack foods/d (median 1.14, 25th-75th percentile 0.71–1.86), with no gender difference. The number of health recommendations met by the same individual was also considered (Table 2.4). About 21% of the sample did not meet any health recommendation, while only about 5% fulfilled three recommendations, and <0.5% fulfilled four recommendations. No gender differences were found.

Finally, healthy lifestyle behaviour and junk snack food consumption were correlated with each other; only significant results are reported here. There was a positive association between having breakfast and eating fruit and vegetables (r=0.113, p<0.02); both of these eating behaviour were positively correlated with milk/yoghurt consumption (r=0.523, p<0.001 and r=0.134, p<0.004, respectively) and negatively correlated with eating junk snack

foods (r=0.173, p<0.001 and r=0.179, p<0.001, respectively). TV viewing was negatively correlated with having breakfast (r=0.093, p<0.05) and eating fruit and vegetables (r=0.154, p<0.01), while it was positively correlated with eating junk snack foods (r=0.186, p<0.001). Total time spent in sedentary behaviour was negatively correlated with eating fruit and vegetables (r=0.181, p<0.001) and positively correlated with eating junk snack foods (r=0.244, p<0.001).

2.3.3 Relationships between meeting health recommendations and abdominal adiposity

When the association between WHtR as outcome variable with each of the healthy lifestyle behaviour as independent variables was considered, the only positive relationship emerged with TV viewing (r=0.118, p<0.05). On the other hand, as the number of healthy recommendations met decreased, the percentage of adolescents with abdominal adiposity rose (Fig.2.1, p<0.002, χ^2 for trend). The trend was not significant for the prevalence of OW/ OB (p=0.329). Having regular breakfast, performing \geq 60 min of MVPA/day and watching TV for <2 h/day represented the most frequent cluster of healthy lifestyle behaviour (89 %) in adolescents with WHtR<0.5. Finally, the logistic regression model demonstrated that male gender (OR 2.560, 95% CI 1.338, 4.892; p<0.005) and watching TV for \geq 2 h/day (OR 2.260, 95% CI 1.154, 4.427; p<0.02) were the only independent variables positively associated with high WHtR values.

2.4 DISCUSSION

The main results of the present study indicated that: adolescents living in the Campania region only seldom meet health recommendations on diet and physical activity; healthy behaviours are correlated with each other; and there is a positive association of male gender and TV viewing with abdominal adiposity.

The present study has considered main dietary and physical activity recommendations as important determinants of present and future health (Crespo *et al*, 2001; Edwards *et al*, 2011; Ross, 2011; Strong *et al*, 2005).

First, the proportion of adolescents meeting each of the recommendations was evaluated.

Skipping breakfast has been related in adolescence to irregular pattern of meals and junk food consumption (Affenito *et al*, 2005; Hoyland *et al*, 2009; Kant *et al*, 2008), and it is also associated with an increased risk of excess body fat (Szajewska and Ruszczynski, 2010). Consistent with previous studies (Hallström *et al*, 2011; Mazzarella and Pizzuti, 2011), breakfast was consumed at least 6 days/week only by 55% of our sample and more frequently by boys than girls, as already observed in northern Italy and throughout Europe (Pearson *et al*, 2009; Vanelli *et al*, 2005).

The consumption of fruit and vegetables is commonly promoted because they are nutrient-dense foods with a potential effect, even if relatively small, in protecting against adiposity in children (Davis *et al*, 2007). In agreement with previous surveys (Larson *et al*, 2007; Mazzarella and Pizzuti, 2011) the average consumption of fruit and vegetables was very low in the adolescents studied, with only a low percentage of them (2.9%) consuming at least 5 servings/day.

Another marker of a healthy diet is eating non-energy-dense dairy products (ADA, 2007), which are an important source of calcium (Ross, 2011). In our sample only 1.9% of the adolescents met the guidelines for milk and yoghurt consumption, in line with previous data regarding our region (Mazzarella and Pizzuti, 2011).

In addition to healthy dietary behaviour, lifestyle recommendations for adolescents promote physical activity and the reduction of sedentary behaviour, aiming for better physical fitness and psychological health (Strong et al, 2005). In particular, low physical activity and excessive TV viewing are both considered in adolescents as significant determinants of excess body fat and cardio-metabolic risk factors (Crespo et al, 2001; Strong et al, 2005). Physical activity was evaluated using a well-established questionnaire (IPAQ) (Hagströmer et al, 2008; Strong et al, 2005). A threshold of 60 minutes of MVPA/day, chosen according to evidence-based recommendations (WHO, 2010b), was met by 13.6% of our sample with no gender difference. This value was slightly higher than that reported by the HBSC regional survey (8.1%) (Mazzarella and Pizzuti, 2011), possibly because of the different methodology. On the other hand, similar results have been obtained, as compared with the HBSC regional survey (Mazzarella and Pizzuti, 2011), with respect to the percentage of adolescents (<50%) meeting the recommendation for TV viewing.

Finally, the consumption of junk snack foods (sweet and savoury products) was also specifically assessed, because of its negative impact on diet quality (Capewell and McPherson, 2010). Quite astonishingly, >65% of our sample ate at least 1 serving of junk snack foods/day.

As far as is known, few papers have focused on the clustering of dietary, physical activity and sedentary behaviour in adolescents. First, we considered the selected health behaviour as dichotomized variables (meeting or not meeting health recommendations): only less than one adolescent out of twenty met three or four recommendations, with the most prevalent cluster represented by having breakfast, performing physical activity and limiting

TV viewing; while, on the other hand, more than one adolescent out of five did not meet any health recommendation.

In a similar study on patterns of nutrition and physical activity behaviour in adolescents, Sanchez et al. (2007) reported that only 2% of Spanish adolescents completely met the guidelines for diet (fat <30% of total energy and fruit/ vegetables >5 servings/day), physical activity (>60 min/day) and sedentary healthy behaviour (TV<120 min/day). Second, the five health behaviour (having breakfast, eating fruit and vegetables, consumption of milk/yoghurt, performing MVPA, limiting TV viewing) were related to each other and with junk snack food consumption. There were significant correlations between the three dietary behaviour. TV viewing was inversely related to having breakfast or eating fruit and vegetables, while junk snack food intake not only negatively correlated with breakfast frequency, but was also positively related to TV viewing, possibly because of concurrent eating and/or exposure to food advertising (Council on Communications and Media, 2011). Although a direct comparison with previous data cannot be performed, since the behaviour considered were not strictly the same, a few studies have already confirmed that risk-related behaviour may coexist in young adolescents (Dumith et al, 2012; Pearson and Biddle, 2011; Seghers and Rutten, 2010). Meeting health recommendations and abdominal adiposity WC, which is a marker of abdominal fat (Savva et al, 2000), has been shown to be related to having breakfast (Alexander et al, 2009), fruit and vegetable consumption (Bradlee et al, 2010), physical activity (Klein-Platat et al, 2005) and sedentary behaviour (Martinez-Gomez et al, 2010). In addition, mounting evidence has demonstrated the benefits of regular physical activity as treatment for abdominal obesity in association with energy restriction (Kim and Lee, 2009).

As far as we are aware, the present study is the first one that has evaluated and clearly demonstrated the association between meeting health recommendations and abdominal adiposity. As the number of health recommendations met decreased, the proportion of adolescents with abdominal adiposity increased (Fig. 2.1), while no significant trend was observed for the prevalence of OW/OB.

Taking into consideration also the above-mentioned relationships between TV viewing and dietary behaviours, such as consumption of junk snack foods (Council on Communications and Media, 2011; Francis *et al*, 2003; Harrison and Marske, 2005; Matheson *et al*, 2004), it is not surprising that the logistic regression analysis model also identified TV viewing as an independent predictor of WHtR in our adolescents. On the contrary, we failed to demonstrate any association between total screen time (TV viewing, video game and computer use) and WHtR (r=0.05), possibly because of the specific behavioural setting of the Neapolitan area (e.g. less time spent using computers).

Overall, the results of the present study are likely to depend on the criteria chosen for defining recommendations. Some healthy behaviour, such as having breakfast and limiting TV watching, more probably entered in the cluster of recommendations met just because they were the most prevalent. Thus, the results of the logistic regression analysis model cannot be interpreted in the sense that the role of fruit and vegetable consumption or MVPA in the prevention of abdominal adiposity should be denied.

We should acknowledge that the study has some limitations such as its crosssectional design, the relatively narrow age range and the restriction to the urban setting in one southern region of Italy. On the other hand, the strengths of the study are that all data were collected by interview and carefully checked by trained nutritionists rather than self-reported; in addition, all anthropometric data were carefully measured rather than being reported.

2.5 CONCLUSIONS

The WHO recommends the implementation of strategies in order to face the multiple risks linked to unbalanced diet and sedentary lifestyle. Our study indicates that adolescents not often meet multiple health recommendations on diet and physical activity, confirming that there is a strong need for effective strategies to promote healthy behaviour during adolescence. In particular, watching TV for ≥ 2 h/day is the marker more strictly related to abdominal fat in adolescence.

We suggest that the calculation of WHtR should be included in health surveillance systems, because not only it is a predictor of cardiovascular risk but also it is sensitive to the effect of unhealthy lifestyle.

	Total	(n 478)	Boys (n 204)		Girls		
	Median	IQR	Median	IQR	Median	IQR	р
Age (years)	16.3	15.6-16.7	16.0	15.4-16.8	16.4	15.9-16.8	< 0.001
Height (cm)	165.0	159.4- 172.2	173.7	168.9-178.5	160.6	157.1-165.0	< 0.001
Weight (Kg)	63.3	55.9-72.4	69.3	61.4-80.4	58.5	52.9-66.0	< 0.001
BMI (Kg/m ²)	22.8	20.8-25.6	23.0	21.0-25.9	22.7	20.6-25.1	0.170
WC (cm)	72.0	67.4-77.8	77.5	71.6-83.8	69.6	65.5-73.7	< 0.001
WHtr	0.43	0.41-0.47	0.44	0.41-0.49	0.43	0.41-0.46	< 0.050

Table 2.1: Anthropometric characteristics of the study population

IQR: 25th-75th percentile

	n	%	n	%	n	%	Р
Father's educational level							0.757
Elementary	17	3.6	9	4.4	8	2.9	
Middle School	117	24.5	52	25.5	66	24.1	
High school	208	43.5	88	43.1	119	43.4	
Degree	136	28.4	55	27.0	81	29.6	
Mather's educational level							0.202
Elementary	26	5.4	13	6.4	13	4.7	
Middle School	106	22.2	52	25.5	54	19.7	
High school	230	48.1	87	42.6	143	52.2	
Degree	116	24.3	52	25.5	64	23.4	

Table 2.2: Educational level of the parents of 478 adolescents

	Total	(n 478)	78) Boys (n		Girls	(n 274)	р
	n	%	n	%	n	%	
Breakfast ≥6 days/ week	265	55.4	124	60.8	141	51.5	0.042
Fruit/veg ≥ 5 servings/week	14	2.9	8	3.9	6	2.2	0.267
Milk/yoghurt ≥ 3 servings/day	9	1.9	5	2.5	4	1.5	0.012
MVPA ≥ 60 min/day	65	13.6	26	12.7	39	14.2	0.639
TV viewing < 2h/day	221	46.3	83	40.7	138	50.4	0.036

Table 2.3: Proportion of the study population who met each of the selected health recommendations.

International recommendations used in children and adolescents for a healthy nutrition and lifestyle: 1) Breakfast \geq 6days/week; 2) \geq 5 serving of fruit and vegetables/day; 3) \geq 3 servings/day of milk and/or yoghurt; 4) \geq 60 minutes/day of Moderate to Vigorous Physical Activity; 5) < 2 hours/day of Television viewing.

	Total (n 478)		Boys	(n 204)	Girls (n 274)		
Number of health recommendations met	n %		n	%	n	%	
0	103	21.5	38	18.6	65	23.7	
1	202	42.3	96	47.1	106	38.7	
2	149	31.2	62	30.4	87	31.8	
3	23	4.8	7	3.4	16	5.8	
4	1	0.2	1	0.5	0	0.0	
5	0	0.0	0	0.0	0	0.0	

Table 2.4: Distribution of meeting multiple health recommendations in the study population.

Fig. 2.1: Percentage of cases with abdominal adiposity (WHtr > 0.5 = grey; or overweight/obese = white) in the different categories of multiple healthy recommendations met among the studied 478 adolescents.



CHAPTER 3: SYSTEMATIC REVIEW ON THE MEDITERRANEAN DIET IN CHILDREN AND ADOLESCENTS

3.1 INTRODUCTION AND AIM

The term "Mediterranean Diet" has been widely used to describe the traditional dietary habits of people living around the Mediterranean basin, in particular in the olive tree growing areas, whereas "traditional" means before the globalization expanded also to the food culture.

Mediterranean Diet (MD) is characterized by abundant plant foods (fruits, mainly as typical daily desserts, vegetables, bread, other forms of cereals, beans, nuts and seeds). It also includes olive oil as the principal source of fat, moderate amounts of dairy products (principally cheese and yoghurt), low to moderate amounts of fish, low amounts of poultry and red meat and wine consumed in low to moderate quantities, normally with meals (Kafatos *et al.*, 2000; Willet *et al.*, 1995).

The Mediterranean dietary pattern is characterized as rich in monounsaturated fatty acids, a balanced ratio of (n-6):(n-3) essential fatty acids, and high amounts of fibre and antioxidants, such as vitamins E and C, resveratrol, polyphenols, selenium, and glutathione.

Moreover, seasonality, biodiversity, the use of traditional and local food products are also important elements in this pattern. MD has also qualitative cultural and lifestyle elements, such as frugality, sobriety, conviviality, culinary activities, physical activity and adequate rest (Bach-Faig *et al.*, 2011).

MD is associated with a lower incidence of mortality (Sofi *et al.*, 2010), lower incidence of cardiovascular disease (Estruch *et al.*, 2013), type 2 diabetes (Salas Salvadó *et al.*, 2013), certain types of cancer (Couto *et al.*, 2011) and neuro-degenerative disease (Sofi *et al.*, 2010) in the adult population.

The extremely high prevalence of childhood overweight and obesity has become a major public health issue in developed countries and worldwide (Waters *et al.*, 2011). Obesity often persists into adulthood, increasing children's risk of developing cardiovascular disease, type 2 diabetes and other chronic diseases later in life (Lobstein *et al.*, 2004). There is also good evidence that obesity is associated with high intake of energy-dense, nutrient poor foods such as soft drinks, savory crisps and sweet biscuits, and increased time spent in sedentary behaviour (Carlson *et al.*, 2012; WHO, 2003).

On the other hand, regular physical activity (PA) and a diet high in fruit, vegetables, legumes and whole-grain cereals have been shown to be protective (Swinburn *et al.*, 2004; WHO, 2003).

These are the reasons which induced to analyse the literature on MD in the children and adolescents' population. The purpose of this paper is to review all the studies which analysed the adherence to the MD with a quantifying score, to present the possible predictive factors to the adherence and to evaluate the association made between MD adherence and different variables.

3.2 METHODS

A search of scientific literature was conducted on MEDLINE (National Library of Medicine, Bethesda, MD, USA) and Scopus by 2 independent researchers for relevant articles about the MD in children and adolescents published in the last 20 years, from January 1994 to 30 March 2015. We used the keywords "Mediterranean diet", "children", "adolescents", "index" and "score". We did not limit the search to studies published in English, in order to include possible interesting publications written in the languages of the Mediterranean basin. Additional publications were identified from references provided in original papers. We limited the search to articles regarding humans.

Inclusion criteria

We focused the search on articles referring to the MD and its effect on health in the first two decades of life, on MD adherence through a quantifying score, and we included only population-based papers. Cross-sectional and longitudinal studies were included, while case-control studies excluded. Intervention studies and reviews, editorials and comments were only considered for the discussion. Reviews were also used as a further control, so as to be sure not to exclude any article on the topic of MD in children and adolescents.

3.3 RESULTS

3.3.1 Description of the papers

Based on the inclusion criteria the first search led to 297 potential articles; 206 papers were excluded because they considered adults, were published before 1994, or were not of interest. From the resulting 91 articles, 37 were

excluded because they did not include MD scoring data, and 2 were excluded because they referred to special populations (sport professionals). We finally analyzed 52 articles and 58 cohorts (Figure 3.1). All cohorts are represented by both genders. None of the papers had a study design that could infer causality (Table 3.1).

Country of study

The majority of the studies presented data from the Mediterranean basin (Greece 22, Spain 17, Italy 4, Turkey 1, Cyprus 1). Moreover, there were 2 articles on Portuguese data, 2 on British data and 1 on Mexican data. Additionally, 2 articles were multi-centric studies on European countries.

Subject age

Eight articles presented data exclusively on children in their first decade, 32 papers presented data only on adolescents (second decade), 5 papers presented data separately on children and adolescents, while 7 articles showed data on a mixed population of children and adolescents.

Year of publication

No articles were published before 2003 with the specified inclusion criteria. A single article was published in 2003, 1 paper was published in 2004, 3 in 2007, 4 in 2008, 6 in 2009, 8 in 2010, 11 in 2011, 3 in 2012, 8 in 2013 and 9 in 2014. In 2015, so far 1 article has been published.

3.3.2 Methods for the evaluation of the adherence to the Mediterranean Diet

Among children and adolescents, adherence to the Mediterranean Diet is usually assessed through the KIDMED index, but other indexes have also been used.

Irrespective of how conformity to the Mediterranean diet is computed, all scores share some common ground; they are all based on the dietary

components that capture the essence of this dietary profile. In particular, 4 different tools for evaluating MD adherence were identified in the reviewed papers: the KIDMED index, some modified versions of Thricopulou's Mediterranean Diet Score (Thricopoulou *et al.*, 1995; Thricopoulou *et al.*, 2003), Psaltopoulou' Mediterranean Diet Score (Psaltopoulou *et al.*, 2004), and a food frequency based Mediterranean Diet Score (fMDS).

More specifically, 7 studies used Thricopoulou's MDS and its different modified versions (De Battle *et al.*, 2008; del Mar Bibiloni *et al.*, 2011; del Mar Bibiloni *et al.*, 2012b; Jennings *et al.*, 2011; McCourt *et al.*, 2014; Noale *et al.*, 2014; Santos *et al.*, 2014), 3 studies (Mariscal-Arcas *et al.*, 2010; Martinez *et al.*, 2010; Ozen *et al.*, 2015) used the Sanchez-Villega method based on z-values (Sanchez-Villega *et al.*, 2002), 3 studies (Castro-Rodriguez *et al.*, 2008; Garcia-Marcos *et al.*, 2007; Gonzales-Barcalà *et al.*, 2010) used the modified version of the method suggested by Psaltopoulou *et al.* (2004) and Tognon *et al.* (2014a and 2014b) used the fMDS.

Because the above 15 studies, which used different methods from the KIDMED index, present their results in a non-comparable way, and in any case, the adherence categories are expressed as inner median values, it has been decided not to present the adherence level for these populations, but only for those evaluated through the KIDMED index.

3.3.3 Adherence to the Mediterranean Diet through the KIDMED index

Thirty seven studies evaluated the adherence to the MD through the KIDMED index (20 of them used the KIDMED questionnaire, while the other 17 used a general food frequency questionnaire, from which the KIDMED score was extrapolated). Among them, 3 authors (Chatzi *et al.*,

2007; Monjardino *et al.*, 2014; Roccaldo *et al.*, 2014) used a slightly modified version of the original KIDMED, removing 1 up to 4 questions. In particular, Chatzi *et al.* (2007) excluded the items on the consumption of fast foods, sweets, and legumes since this information was not requested in the diet questionnaire used in the study. Monjardino *et al.* (2014) eliminated the items regarding breakfast since the dietary evaluation was based on an FFQ. Roccaldo *et al.* (2014) did not include weekly frequency of fast food visits because fast food restaurants were not uniformly distributed in all the Italian areas investigated.

Fourteen studies expressed the results as KIDMED score mean. The minimum KIDMED score mean was found by Farajan *et al.* (2011) and was 3,65 \pm 2,27; the maximum from Perez Gallardo *et al.* (2011) and was 7,6 \pm 2,0. The mean is 6,5.

Twenty eight studies expressed their results dividing the sample into the 3 KIDMED categories (poor, average, good) suggested by Serra-Majem *et al.* (2004). The poor category varied from 1,6% in a sample of Spanish children (Mariscal-Arcas *et al.*, 2009) to 62,8% in a sample of Greek 10-12 years old subjects (Magriplis *et al.*, 2011). The mean poor category is 25%. The average category varied from 28% in a sample of Greek adolescents (Tsartsali *et al.*, 2009) to 69,9% in a Greek population of children and adolescents (Kontogianni *et al.*, 2008). The mean average category is 52%. The good category varied from 2,4% in a Greek urban population (Grigoropoulou *et al.*, 2011) to 53,9% in Spanish children (Perez Gallardo *et al.*, 2011). The mean good category is 21,2%.

Finally, 11 authors described the adherence to MD by both the KIDMED score mean and the division in categories.

3.3.4 Potential predictive factors associated with adherence to the Mediterranean Diet

Gender

Twenty eight studies (Arriscado *et al.*, 2014; Bargiota *et al.*, 2013; del Mar Bibiloni *et al.*, 2012b; Farajian *et al.*, 2011; Grao-Cruces *et al.*, 2013; Grao-Cruces *et al.*, 2014; Grosso *et al.*, 2013; Jennings *et al.*, 2011; Kontogianni *et al.*, 2008; Lazarou *et al.*, 2009a; Lazarou *et al.*, 2009d; Magriplis *et al.*, 2011; Mariscal-Arcas *et al.*, 2010; Martinez *et al.*, 2010; Mazaraki *et al.*, 2011; McCourt *et al.*, 2014; Monjardino *et al.*, 2014; Noale *et al.*, 2014; Ozen *et al.*, 2015; Perez Gallardo *et al.*, 2011; Roccaldo *et al.*, 2014; Sahingoz and Sanlier, 2011; Santomauro *et al.*, 2014; Serra-Majem *et al.*, 2004; Tognon *et al.*, 2014b; Tsartsali *et al.*, 2009; Vassiloudis *et al.*, 2014) analysed the association between adherence to MD and gender.

Six of the 28 cited studies found a significant difference between genders, showing (5 out of 6) that girls had a higher level of adherence then boys.

Age

Twenty one studies (Arriscado *et al.*, 2014; Ayechu and Durà, 2010; Bargiota *et al.*, 2013; del Mar Bibiloni *et al.*, 2011; Farajian *et al.*, 2011; Gonzales-Barcalà *et al.*, 2010; Grao-Cruces *et al.*, 2013; Kontogianni *et al.*, 2008; Lazarou *et al.*, 2009d; Mariscal-Arcas *et al.*, 2009; Mariscal-Arcas *et al.*, 2010; Martinez *et al.*, 2010; Mazaraki *et al.*, 2011; McCourt *et al.*, 2014; Noale *et al.*, 2014; Ozen *et al.*, 2015; Schröder *et al.*, 2010; Serra-Majem *et al.*, 2004; Tognon *et al.*, 2014a; Tognon *et al.*, 2014b; Tsartsali *et al.*, 2009) analysed the association between adherence to MD and age.

Seven of the 21 cited studied found a detrimental trend in MD adherence with growth, which was significant in 5 studies. Only one Spanish study (Ayechu and Durà, 2010) found an opposite trend.
Socio-economic status

SES was evaluated in different forms: 1 study evaluated various indexes of economic level as owning more than one vehicle and children with their own bedroom; 7 studies evaluated the parents educational level, 4 studies observed the type of parents' occupation, and 8 studied evaluated the socio-economic level through the parents education plus their occupation.

Seventeen studies (Antonogeorgos *et al.*, 2013; Arriscado *et al.*, 2014; Bargiota *et al.*, 2013; del Mar Bibiloni *et al.*, 2012b; Kontogianni *et al.*, 2008; Lazarou *et al.*, 2009d; Martinez *et al.*, 2010; Monjardino *et al.*, 2014; Ozen *et al.*, 2015; Roccaldo *et al.*, 2014; Sahingoz and Sanlier, 2011; Santomauro *et al.*, 2014; Santos *et al.*, 2014; Schröder *et al.*, 2010; Serra-Majem *et al.*, 2004; Tognon *et al.*, 2014b; Tsartsali *et al.*, 2009) analysed the association between MD and socio-economic status (SES).

Two authors (Antonogeorgos *et al.*, 2013; del Mar Bibiloni *et al.*, 2012b) studied the relationship between SES and MD adherence as primary outcome.

Thirteen of the 17 studied found a significant positive correlation between adherence to the MD and SES. In particular, 6 studies (Bargiota *et al.*, 2013; Roccaldo *et al.*, 2014; Sahingoz and Sanlier, 2011; Santomauro *et al.*, 2014; Schröder *et al.*, 2010; Serra-Majem *et al.*, 2004) found that a good adherence to MD is associated with the educational level of the mother.

Geographical setting (urban/rural)

Five studies (Grao-Gruces *et al.*, 2013; Grigoropoulou *et al.*, 2011; Grosso *et al.*, 2013; Lazarou *et al.*, 2009b; Lazarou *et al.*, 2009d) analysed the association between adherence to MD and rural/urban setting. While in 2 cases the association was not significant, 3 authors (Grao-Gruces *et al.*, 2013;

Grigoropoulou *et al.*, 2011; Grosso *et al.*, 2013) found a positive correlation between the rural setting and the adherence to MD, suggesting that in rural areas people still live and eat in a more "traditional" way, far from junk and fast food.

3.3.5 Association between adherence to the Mediterranean Diet and anthropometric or body composition variables

A total of 24 studies were identified from those initially meeting all the search criteria (Table 3.2); four of them, namely two articles by Kontogianni *et al.* (2008 and 2010) and two by Lazarou *et al.* (2009d and 2010) were performed on the same population, but analysed different variables. According to the study type, 21 studies were cross-sectional and 3 were both cross-sectional and prospective (McCourt *et al.*, 2014; Monjardino *et al.*, 2014; Tognon *et al.*, 2014b); in particular Tognon *et al.* (2014b) performed a baseline survey that was followed by an intervention phase repeated after two years. The intervention was focused on diet, physical activity and stress-coping capacity; it was conducted in selected regions in each country. A similar region with no obesity prevention strategies implemented, served as the control population.

Characteristics of the study sample

The studies were carried out between 2007 and 2014 in seven different countries, while one study (Tognon *et al.*, 2014b) was multi-centric, involving 8 European countries (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium, Estonia). The majority of the studies (20 out of 24) were from Mediterranean countries (Italy, Spain, Cyprus, Greece), while only 3 studies were from non-Mediterranean countries (Britain, Portugal and Northern Ireland) (Jennings *et al.*, 2011; McCourt *et al.*, 2014). As a whole,

the studies involved 65,292 subjects (the minimum sample size was 200, the maximum was 20,106 individuals).

Five studies reported data on children in their first decade, while 16 studies reported data on adolescents/young adults in their second decade. In addition, two cross-sectional studies (Kontogianni *et al.*, 2008; Kontogianni *et al.*, 2010) and one prospective study (Tognon *et al.*, 2014b) provided separate data on children and adolescents. Finally, 8 studies reported data on children in their first decade, while 19 studies reported data on adolescents/young adults in their second decade.

Only 12 studies (50% of the studies) reported the association between adherence to MD and anthropometric or body composition measures as the predetermined, primary outcome.

Anthropometric data (height, weight, waist circumference) were measured in 15 studies, while they were self-reported (Grosso *et al.*, 2013; Kontogianni *et al.*, 2008; Kontogianni *et al.*, 2010; Noale *et al.*, 2014; Santomauro *et al.*, 2014;) or parent-reported (Garcia-Marcos *et al.*, 2007; Kontogianni *et al.*, 2008; Kontogianni *et al.*, 2010; Lazarou *et al.*, 2009d; Lazarou *et al.*, 2010) in 8 studies. All the 24 studies had height and weight data in order to calculate BMI; 18 also classified weight categories (16 according to IOTF, 1 according to WHO (Ozen *et al.*, 2015), and 1 according to Cacciari *et al.*, (2006) (Noale *et al.*, 2014). Six studies measured fat mass: 4 by bio-impedance analysis (Farajian *et al.*, 2011; Grao Cruces *et al.*, 2014; Jennings *et al.*, 2011; Magriplis *et al.*, 2011) and 2 by skinfold thickness measurement (Arriscado *et al.*, 2014: Tognon *et al.*, 2014a).

The KIDMED index was used to evaluate the degree of adherence to MD in 18 studies, although in 7 cohorts (Arvaniti *et al.*, 2011a-b; Lazarou *et al.*,

2009d; Lazarou *et al.*, 2010; Magriplis *et al.*, 2011; Roccaldo *et al.*, 2014; Schröder *et al.*, 2010; Monjardino *et al.*, 2014) the KIDMED index was extrapolated from a semi-quantitative food frequency questionnaire, not using the KIDMED questionnaire itself. Other questionnaires, specifically designed to assess MD and providing an MD score (MDS) (mainly from Thricopolou *et al.*, 2003) were used in the remaining 6 studies (Garcia Marcos *et al.*, 2007; Jennings *et al.*, 2011; Mc Court *et al.*, 2014; Noale *et al.*, 2014; Ozen *et al.*, 2015; Tognon *et al.*, 2014a).

Cross-sectional studies

Eleven (47.8%) out of 23 cross-sectional studies reported no significant association between adherence to MD and BMI (Arriscado *et al.*, 2014; Arvaniti *et al.*, 2011b; Farajian *et al.*, 2011; Grao-Cruces *et al.*, 2013; Kontogianni *et al.*, 2008; Kontogianni *et al.*, 2010; Jennings *et al.*, 2011; Magriplis *et al.*, 2011; Monjardino *et al.*, 2014; Noale *et al.*, 2014; Tsartsali *et al.*, 2009); two of these studies expressed BMI as BMI z-score in order to make comparisons between different age-groups (Grao-Cruces *et al.*, 2013; Jennings *et al.*, 2011).

Seven studies reported no association with weight categories (Arriscado *et al.*, 2014; Arvaniti *et al.*, 2011b; Farajian *et al.*, 2011;; Jennings *et al.*, 2011; Kontogianni *et al.*, 2010; Magriplis *et al.*, 2011; Roccaldo *et al.*, 2014) and 3 studies with waist circumference (Arriscado *et al.*, 2014; Farajian *et al.*, 2011; Jennings *et al.*, 2011). With regard to body composition, 5 studies found no association between MD adherence and % fat mass (Arriscado *et al.*, 2014; Farajian *et al.*, 2011; Grao-Cruces *et al.*, 2013; Jennings *et al.*, 2011; Magriplis *et al.*, 2011).

On the contrary, the association was significant in 12 studies (52.2%). Higher adherence to MD was associated with lower BMI in 4 studies (Kontogianni

et al., 2010; Lydakis *et al.*, 2012; Perez Gallardo *et al.*, 2011; Schröder *et al.* 2010, lower waist circumference in 2 studies (Lydakis *et al.*, 2012; Schröder *et al.* 2010), and lower % fat mass in one study (Tognon *et al.*, 2014b). With regard to studies analyzing BMI categories, adherence to MD was associated with lower risk of overweight or obesity in 7 studies (Garcia Marcos *et al.*, 2007; Grosso *et al.*, 2013; Kontogianni *et al.*, 2010; Lazarou *et al.* 2009d; Lazarou *et al.*, 2010; Ozen *et al.*, 2015; Tognon *et al.*, 2014b) It should be specified that in three of these studies, obesity status was associated with MD only in specific groups: in girls but not in boys (Lazarou *et al.*, 2009d; in adolescents but not in children (Kontogianni *et al.*, 2010) and in non-functional food consumers (Ozen *et al.*, 2015).

Contrary to these studies, only Mazaraki *et al.* (2011) found an association between higher BMI and WC and high MD adherence in adolescents.

Interestingly, two studies reported data on the association between MD adherence and underweight status (Ozen *et al.*, 2015; Santomauro *et al.*, 2014). In particular, Ozen *et al.* (2015) reported that underweight adolescents who consumed functional food had a higher risk of low MD adherence, while the opposite trend was observed among functional food non-consumers. Analogously, Santomauro *et al.* (2014) reported that the prevalence rate of poor adherence to MD was significantly higher in underweight students (35.9%), while being overweigh or obese was protective against poor adherence to MD.

With regard to age, 4 out of 8 cross-sectional studies including children in their first decade (50%) (Garcia Marcos *et al.*, 2007; Grosso *et al.*, 2013; Perez Gallardo *et al.*, 2011; Tognon *et al.*, 2014b) and 10 out 18 cross-sectional studies including subjects in their second decade (55.5%) (Grosso *et al.*, 2013; Kontogianni *et al.*, 2010; Lazarou 2009d Lazarou *et al.*, 2010

Lydakis *et al.*, 2012; Mazaraki *et al.*, 2011; Ozen *et al.*, 2015; Santomauro *et al.*, 2014; Schröder *et al.*, 2010; Tognon *et al.*, 2014b) reported positive associations between adherence to MD and anthropometric and or body composition data. The study by Kontogianni *et al.* (2010), which provided data divided into age groups from both decades, reported a positive association only in adolescents. McCourt *et al.* (2014) did not provide any anthropometric assessment in the cross-sectional arm of the study.

Prospective or intervention studies

One prospective study found no meaningful association between tertiles of adherence to the MD pattern and mean BMI in 1023 adolescent students, aged 13 years, who were re-evaluated after 4 years of follow-up (Monjardino *et al.*, 2014).

Another study by McCourt *et al.* (2014) analyzed traditional CVD risk biomarkers, including BMI, and adherence to MD in 12–15-year olds (YH1) and again in the same individuals at 20–25 years of age (YH3). They found no longitudinal relationship between the change in the MDS and the change in BMI from YH1 to YH3.

In the intervention study by Tognon *et al.* (2014b) the prospective associations of baseline MDS with the highest sex-specific and age-specific quintiles of change of different anthropometric outcomes (BMI, BMI z-scores, waist circumference, WtHR and percent fat mass) were analyzed after 2 years of follow up by logistic regression analysis, adjusted for different confounding factors (sex, baseline age, study center, parental income and education, baseline age-specific and sex-specific BMI z-scores or waist circumference). High adherence expressed as MDS at baseline protected against increases in BMI (OR=0.87, 95% CI: 0.78; 0.98), WC (OR=0.87, 95% CI: 0.77; 0.98) and WtHR (OR=0.88, 95% CI: 0.78; 0.99)

with a similar trend observed for percent fat mass (p=0.06). Results from all models were unchanged when adjusting for a variable describing inclusion in the intervention study.

3.3.6 Association between adherence to the Mediterranean Diet and lifestyle variables

A total of 23 studies were identified from those initially meeting all the search criteria, however 4 studies were excluded, since the lifestyle variables were only used as covariates in multiple regression analysis (Garcia Marcos *et al.*, 2007; Kontogianni *et al.*, 2010; Lazarou *et al.*, 2010, Tognon *et al.*, 2014b,). Therefore only 19 studies were considered (Table 3.3). According to the study type, all the studies were cross-sectional, including the articles by Monjardino *et al.* (2014) and McCourt *et al.* (2014) (both defined as cross-sectional and prospective), which reported an association between adherence and lifestyle only in the cross-sectional arm.

Characteristics of study samples

The studies were carried out between 2008 and 2014 in 7 different countries. As a whole, they involved 28,075 subjects (the minimum sample size was 321, the maximum was 4,786 individuals). The majority of the studies (14 out of 17) were from Mediterranean countries (Italy, Spain, Cyprus, Greece), while only 3 studies were from non-Mediterranean countries (Great Britain, Portugal and Northern Ireland) (Jennings *et al.*, 2011; McCourt *et al.*, 2014)).

Three studies reported data on children in their first decade of life (2-10 years) (Jennings *et al.*, 2011; Kontogianni *et al.* 2008, Roccaldo *et al.*, 2014); 15 on the second decade of life (10-20 years) (Arriscado *et al.*, 2014; Arvaniti *et al.*, 2011b; Farajian *et al.*, 2011; Grao-Cruces *et al.*, 2013; Grosso *et al.*, 2013; Kontogianni *et al.* 2008; Lazarou *et al.*, 2009d; Magriplis *et al.*,

2011; Mazaraki *et al.*, 2011; McCourt *et al.*, 2014; Monjardino *et al.*, 2014; Noale *et al.*, 2014; Ozen *et al.*, 2015; Santomauro *et al.*, 2014; Schröder *et al.*, 2010.

Only 3 studies reported the association between adherence to the MD and lifestyle behaviour as predetermined, primary outcome (Grao-Cruces *et al.*, 2013; Kontogianni *et al.*, 2008; Santomauro *et al.*, 2014).

Physical activity was assessed through a questionnaire in 16 studies (Arriscado *et al.*, 2014; Arvaniti *et al.*, 2011b; Farajian *et al.*, 2011; Grao-Cruces *et al.*, 2013; Grosso *et al.*, 2013; Kontogianni *et al.* 2008, Lazarou *et al.*, 2009d; Magriplis *et al.*, 2011; Mazaraki *et al.*, 2011; McCourt *et al.*, 2014; Monjardino *et al.*, 2014; Noale *et al.*, 2014; Ozen *et al.*, 2015; Roccaldo *et al.*, 2014; Santomauro *et al.*, 2014; Schröder *et al.*, 2010) while an objective tool to measure PA (accelerometer) was employed in the remaining study (Jennings *et al.*, 2011). Sedentary behaviour, as represented by Television and/or personal computer and/or videogames, were assessed through a questionnaire in 9 studies (Arriscado *et al.*, 2014; Arvaniti *et al.*, 2011b; ; Grao-Cruces *et al.*, 2013; Kontogianni *et al.*, 2008; Lazarou *et al.*, 2009d; Mazaraki *et al.*, 2011; Noale *et al.*, 2014; Roccaldo *et al.*, 2014; Santomauro *et al.*, 2014; Roccaldo *et al.*, 2014; Santomauro *et al.*, 2014; Roccaldo *et al.*, 2014; Santomauro *et al.*, 2013; Kontogianni *et al.*, 2008; Lazarou *et al.*, 2009d; Mazaraki *et al.*, 2011; Noale *et al.*, 2014; Roccaldo *et al.*, 2014; Santomauro *et al.*, 2014). Arriscado *et al.*, 2014) also measured aerobic fitness through the 20-m Shuttle Run test.

Cross-sectional studies

Sixteen studies provided results on PA, since the article by Roccaldo *et al.* (2014) did not show any results regarding PA. Eleven studies (68.75%) (Arriscado *et al.*, 2014; Farajian *et al.*, 2011; Grao-Cruces *et al.*, 2013; Grosso *et al.*, 2013; Jennings *et al.*, 2011; Magriplis *et al.*, 2011; Mazaraki *et al.*, 2011; Monjardino *et al.*, 2014; Santomauro *et al.*, 2014; Schröder *et al.*, 2010; Tognon *et al.*, 2014b) reported a significant association between higher

adherence to the MD and high levels of PA, while only 4 studies (25%) did not report any association (Arvaniti *et al.*, 2011b; Kontogianni *et al.*, 2008; Noale *et al.*, 2014; Ozen *et al.*, 2015).

Eight studies provided results on sedentary behaviour, since the article by Arvaniti *et al.* (2011b) did not show any results regarding sedentary behaviour: seven out of 8 studies (87.5%) (Arriscado *et al.*, 2014; Grao-Cruces et al., 2013; Kontogianni *et al.*,2008; Lazarou *et al.*, 2009d, Mazaraki *et al.*, 2011; Roccaldo *et al.*, 2014; Santomauro *et al.*, 2014) reported a significant association between higher adherence to the MD and less time spent in sedentary behaviour, while only Noale *et al.* (2014) did not report any significant association.

Arriscado *et al.* (2014) reported a significant increase in aerobic capacity in groups with high adherence to the MD (p=0.012); greater adherence to the MD was weakly associated with improved aerobic capacity (r=0.20).

3.3.7 Association between adherence to the Mediterranean Diet and nutrients and eating patterns

Fourteen studies evaluated the association between adherence to the MD with eating habits, food groups, nutrients and energy intake (Table 3.4).

Arvaniti *et al.* (2011a), aiming to evaluate the association of salty-snack consumption and TV viewing, as well as their synergistic role, with the presence of asthma symptoms in 700 Greek adolescents, showed a negative association between the consumption of salty snacks with adherence to the MD. Finally, the analysis revealed that greater adherence to the MD was also associated with a lower likelihood of showing asthma symptoms.

Grosso *et al.* (2013), aiming to examine the factors associated with adherence to the MD in a sample of Sicilian adolescents, found a significant inverse correlation between the KIDMED score and saturated fatty acids (β =-0.099, p<0.001) and a direct correlation with carbohydrates (β =0.004, p<0.001) and fibre (β =0.057, p<0.001). Among the food groups, consumption of sweets (β =-0.008, p<0.001), fast foods (β =-0.009, p<0.001), chips (β =-0.007, p<0.05) and soft drinks (β =-0.003, p<0.001), were inversely correlated with the KIDMED score, whereas a direct correlation with fruit (β =0.010, p<0.001), vegetables (β =0.011, p<0.001), pasta (β =0.019, p<0.001), fish (β =0.029, p<0.001) and cheese (β =0.021, p<0.001) was found.

Jennings *et al.* (2010), analyzing the quality of nutrition in a child sample, used quintiles of MDS to evaluate adherence to the MD. Energy intake was higher in quintile 5 (7330 kJ) compared to quintile 1 (7254 kJ; p=0.005).

Kontogianni *et al.* (2008) exploring the factors affecting MD adherence in a representative sample of Greek children and adolescents, found that KIDMED scores were correlated with the intake of calcium (r=0.187; p<0.001), vitamin C (r=0.146; p<0.001) and fibre (r=0.073; p=0.010).

Kontogianni *et al.* (2010) aimed to identify the clustering of several eating and physical activity habits and behaviour and to explore their potential association with BMI. The authors revealed that a pattern characterized by higher eating frequency, breakfast consumption and a higher KIDMED score, was negatively associated with BMI, after adjustment for age, sex and parental education.

The principal objective of Lazarou *et al.* (2009d) was to evaluate the quality of Cypriot children diet by assessing the degree of adherence to the MD and to examine how the intake of various food groups varies with different levels

of adherence. Compared with the low adherers, those with higher adherence to the MD consumed more dairy products, poultry and rabbit meat, legumes, fish and seafood, nuts, bread, ready-to-eat cereals, whole-grain products, fruit, vegetables, fresh leguminous seasonal vegetables, potatoes (other than fried), olives, low glycaemic index foods, traditional Cypriot food and unrefined foods. It is noted, however, that high MD adherers also reported higher intakes of refined foods and foods high in sugar. After adjustment for potential confounders, such as age, gender, place of residence, BMI, SES and PA, children with at least an average KIDMED score were more likely to eat more frequently seafood and fish, legumes, nuts, bread, fruit, leafy vegetables, olives, low glycaemic index foods and unrefined foods. Children in the "good" KIDMED score category consumed fruit and traditional Cypriot foods more frequently than children in the "average" and "poor" category of KIDMED score.

The main aim of Magriplis *et al.* (2011) was the evaluation of the daily dietary sodium intake of 10-12 year old Greek subjects within the context of the MD pattern. Children with high sodium intake presented better adherence to the MD than participants with low intake. Moreover, increased sodium intake from different food sources was evaluated by the level of adherence to the MD. Children who reported average to good adherence showed a higher sodium intake from the majority of food groups (p<0.001), with the exception of pizza, hamburgers, souvlaki, salty snacks and cakes (p>0.05). Moreover, unadjusted analysis revealed that a 1 unit increase in KIDMED score was associated with a 4-50% increased likelihood of consuming sodium intake above the median value for the majority of foods (p<0.05). These results were confirmed after adjustment for sex, age, BMI and physical activity. More specifically, a 1 unit increase in KIDMED score was associated with a 10% increase in likelihood of consuming total sodium greater than 1500mg/day (which is the EU upper level). The authors

underline, though, that only 4.5% of the children studied showed a good adherence to the MD; therefore the positive association between dietary sodium intake and MD adherence basically concerns children with poor and average adherence to the MD.

Martinez *et al.* (2010) evaluated adherence to the MD among the adolescents in the Balearic Islands through the Mediterranean Dietary Pattern (MDP) proposed by Sanchez-Villega *et al.* (2002) and the factors that might determine good or poor adherence. Youngsters with good adherence to the MDP showed higher consumption of more MD components (cereals and roots, vegetables, fruit, legumes, fish and MUFA:SFA ratio – p=0.001) and lower consumption of meat, whole milk and milk-products (p=0.003) and alcohol (p=0.0016) than adolescents with poor adherence.

Monjardino *et al.* (2014) found that Portuguese adolescents in the highest tertile of adherence to the MD had a significantly higher energy intake and calcium intake compared to those in the first and second tertiles (p<0.001).

Noale *et al.* (2014) aimed to evaluate adherence to the MD in different Italian regions through the MDS (Thricopolou *et al.*, 2003). The authors showed that adolescents whose diets most closely adhered to the MD assumed higher median values of carbohydrates, fibre, vitamin B6, vitamin C, folic acid, vitamin A, vitamin D, iron and monounsaturated fats (p<0.05) and less median values of total saturated fats (p<0.001) and higher MUFA:SFA ratio (p<0.001).

The principal aim of Ozen *et al.* (2015) was the assessment of differences in MD adherence between functional food (FF) consumers and non-consumers. The level of adherence to the MD was assessed by the MDP proposed by Sanchez-Villega *et al.* (2002). The median adherence to the MD for FF

consumers was 56.07%, while that for non-consumers was 55.17%. Average daily intakes of many FF (i.e. fruit juice, fibre-rich bread/cookies, cereal bars, fish and soya milk) and functional dietary components (i.e. dietary fibre, vitamin C, vitamin E, carotene and folic acid) were found to be higher among adolescents with high adherence to the MD than among those with low adherence. Moreover, the intake of vitamin E met the Recommended Dietary Intake only in FF consumers who had high MD adherence.

Schröder *et al.* (2010), aiming to analyse the association between MD adherence and various food groups, found that energy intake was positively associated with the KIDMED index (p<0.001). Higher consumption of fruit and vegetables (p<0.001), cereals, fish, dairy products, protein, and dietary fibre were associated with higher KIDMED scoring. In contrast, consumption of meat (p=0.015), pastry and sweets (p<0.001) decreased across categories of the KIDMED index.

The aim of Schröder *et al.* (2013) was to analyse the association between dietary energy density (DED) with diet quality (measured by the KIDMED score). High DED was associated with a 114% higher risk of low quality diet. Adherence to the MD decreased with the increasing of DED and diets with high energy density were characterized by poor adherence to the MD.

The principal aim of Serra-Majem *et al.* (2003) was the analysis of the nutritional status of 3,166 Spanish individuals aged 6-24, with regard to compliance with the MD. Energy intake did not change according to the KIDMED index, while consumption of fibre, calcium, iron, magnesium, potassium, phosphorus, and practically all the vitamins, with the exception of vitamin E, increased according to the KIDMED index.

Although these studies have many different primary outcomes and analyse different variables, a synthesis it can be made for those studies which considered the same factors.

Two of the three studies which analysed the association between adherence to the MD and energy intake found that adherence to the MD is significantly correlated with higher energy intake, while only one study (Serra-Majem *et al.*, 2003) did not find any correlation.

Six studies analysed the association between adherence to the MD and macro- and micro-nutrients (Grosso *et al.*, 2013; Kontogianni *et al.*, 2008; Monjardino *et al.*, 2014; Noale *et al.*, 2014; Ozen *et al.*, 2015; Serra-Majem *et al.*, 2004). They illustrated how a good adherence to the MD is significantly associated with a high intake of fibre (4 studies), calcium (3 studies), vitamin C (4 studies), iron (2 studies), carbohydrates (2 studies) and a higher MUFA:SFA ratio (3 studies). Moreover, Magriplis *et al.* (2011), who studied the association with sodium intake, found that average and good adherence to the MD is associated with higher sodium intake in Greek adolescents.

Finally, 5 studies analysed the association between adherence to the MD and various food groups. MD adherence has been shown to be positively correlated with a higher intake of fruit, vegetables, fish, legumes, cereals and dairy products, while negatively correlated with fast foods, salty snacks, sweets, soft drinks and meat.

3.4 DISCUSSION AND CONCLUSIONS

Adherence to a Mediterranean Diet can significantly decrease the risk of overall mortality, mortality from cardiovascular diseases, incidence of mortality from cancer, and incidence of Parkinson's disease and Alzheimer's disease in adult populations. These results are also strictly concordant with current guidelines and recommendations from all the major scientific associations that strongly encourage a Mediterranean-like dietary pattern for primary and secondary prevention of major chronic diseases (Sofi *et al.*, 2010). Moreover, epidemiological evidence assessing the association between MD adherence and overweight/obesity is limited and conflicting (Buckland *et al.*, 2008).

The systematic review presented in this paper aimed to analyse the level of adherence to the MD of children and adolescents and its possible predictive factors, its association with adiposity indexes, lifestyle variables and body composition variables and its nutritional adequacy.

Regarding the possible predictive factors, about 25% of the studies which analysed the association between MD adherence and gender found that girls adhere to the MD significantly more than boys, and in 33% of the studies adherence to the MD decreased with age. Moreover, in about 80% of the studies which analysed the association of MD adherence with SES showed a significant correlation between good adherence and parental educational level.

Finally, it seems that living in a rural setting protects from non-adhering to the MD (although only 5 studies analysed this association).

As regards the association between high adherence to the MD and anthropometric or body composition variables, about 50% of the studies that analysed them found significant correlations (lower BMI, lower risk of overweight or obesity).

Concerning the association between adherence to the MD and lifestyle variables, almost 70% of the studies reported a significant association between higher adherence to the MD and high levels of PA, while only 4 studies (25%) did not report any association. Moreover, about 87.5% of the studies (7 out of 8) reported a significant association between higher adherence to the MD and less time spent in sedentary behaviour.

As regards the nutritional adequacy of the MD, it can be concluded that MD adherence is associated with higher intake of fibre, calcium, iron, vitamin C, better MUFA:SFA and lower intake of SFA. Moreover, high adherence to the MD is positively associated with the intake of the most recommended foods for health (fruit and vegetables, legumes, fish, whole grain cereals) and negatively associated with soft drinks, snacks, fast foods and sweets, whose consumption is often very common among children and adolescents.

Although further research is needed, especially on children where the number of published papers is quite scarce, it can be stated that the MD can be a useful tool for the prevention of nutritional inadequacy, overweight and obesity and all related disease in children and adolescents. Figure 3.1: Flow chart of the articles' screening.



First Author	Year of publication	Country of study	Sample size	Age	Main object
Antonogeorgos	2013	Greece	1125	2	association bw MD and OW/OB
Arriscado	2014	Spain	321	2	association bw MD and SES/lifestyle
Arvaniti	2011a	Greece	700	2	association bw TV and snacks with asthma
Arvaniti	2011b	Greece	700	2	association bw MD and asthma
Ayechu	2010	Spain	1956	2	MD adherence
Bargiota	2013	Greece	382	2	food choices analysis
Castro-Rodriguez	2008	Spain	1784	1	association bw MD and wheezing
Chatzi	2007	Greece	690	4	association bw MD and asthma/allergies
Costarelli	2013	Greece	359	2	association bw MD and HRQL
De Battle	2008	Mexico	1476	1	association bw MD and asthma
del Mar Bibiloni	2011	Spain	362	2	association bw MD and MS
del Mar Bibiloni	2012a	Spain	1961	2	association bw MD and sed. behav/lifestyle
del Mar Bibiloni	2012b	Spain	1231	2	MD adherence vs. Western Diet adherence
Farajian	2011	Greece	4786	2	MD adherence + OW/OB prevalence
Garcia-Marcos	2007	Greece	20106	1	association bw MD and OB/asthma/rinoconjuntivitis
Gonzales Barcalà	2010	Spain	14745	3	association bw MD and asthma
Grao-Cruces	2013	Spain	1973	2	MD adherence
Grao-Cruces	2014	Spain	19888	2	association bw MD and fitness/life satisfaction
Grigoropoulou	2011	Greece	1125	2	association bw MD and asthma
Grosso	2013	Italy	1136	2	MD adherence
Jennings	2011	UK	1700	1	association bw MD and diet quality/OW/OB
Kontogianni	2008	Greece	1305	3	MD adherence
Kontogianni	2010	Greece	1305	3	association bw lifestyle patterns and BMI
Lazarou	2009a	Greece	1140	4	lifestyle description
Lazarou	2009b	Greece	1140	4	lifestyle description
Lazarou	2009c	Greece	622	2	association bw MD and blood pressure
Lazarou	2009d	Greece	1140	4	MD adherence

1140

4

association bw MD and OB

Greece

2010

Lazarou

Table 3.1: Main characteristics of 52 articles considered for the review (it continues on the next page).

 Table 3.1: continued.

First Author	Year of publication	Country of study	Sample size	Age	Main object
Lydakis	2012	Greece	277	2	association bw MD and arterial stifness
Magriplis	2011	Greece	4580	2	Identification of sodium intake
Mariscal-Arcas	2010	Spain	3190	3	comparison bw 2 methods to evaluate MD adherence
Mariscal-Arcas	2009	Spain	3190	3	evaluation of KIDMED index
Martinez	2010	Spain	1231	2	MD adherence
Mazaraki	2011	Greece	365	2	association bw MD and albumine/creatinine ratio
McCourt	2014	Northern Ireland	1015	2	association bw MD and CVD
Monjardino	2014	Portugal	1180	2	association bw MD and bone mineral density
Noale	2014	Italy	565	2	MD adherence differences bw different regions
Ozen	2015	Spain	1961	2	MD adherence + consumption functional foods
Perez Gallardo	2011	Spain	350	1	effectiveness of nutritional programmes
Rocaaldo	2014	Italy	1740	1	MD adherence
Sahingoz	2011	Turkey	890	2	comparing nutr habits with knowledge
Santomauro	2014	Italy	1127	2	MD adherence
Santos	2014	Portugal	517	2	SES and metabolic risk factors
Schröder	2010	Spain	2513	2	association bw MD and WC
Schröder	2013	Spain	2513	2	energy density, diet quality, body fat
Serra-Majem	2003	Spain	3166	4	MD adherence and dietary intake
Serra-Majem	2004	Spain	3850	4	development of KIDMED questionnaire
Tognon	2014a	Multicentric	7940	1	Md adherence in Europe
Tognon	2014b	Multicentric	16200	1	association bw MD and OW/OB
Tsartsali	2009	Greece	200	2	association bw MD and knowledge
Tsioufis	2009	Greece	498	2	association bw microalbuminuria and lifestyles
Vassiloudis	2014	Greece	528	2	association bw MD and academic performance/energy balance behaviour

Abbreviations: bw = between; MD = Mediterranean Diet; OW = overweight; OB = obesity; SES = socio-economic status; TV = Television; HRQL = health-related quality of life; MS = Metabolic Syndrome; Sed. behav. = Sedentary Behaviour. Age: 1 = subjects in their first decade of life; 2 = subjects in their second decade of life; 3 =

separated cohorts of children and adolescents; 4 = joint cohort for children and adolescents.

Table 3.2: Association between adherence to the MD and anthropometric and body composition variables in 52 articles considered for the review (it continues on the next page).

First Author/ Year	Anthropometry	OW/OB Definition	Body composition	Main results
Arriscado 2014	measured	NR	ND	NS associations b/w MD adh and BP, BMI, WC.
Arvaniti 2011	measured	IOTF	ND	NS associations b/w MD adh. and PA, BMI, OB.
Farajian 2011	measured	IOTF	BIA	MD adh did not differ b/w NW and OW/OB; BMI, % BF WC did not differ among KIDMED CAT.
Garcia- Marcos 2007	reported by parents	IOTF	ND	KIDMED score differed significantly b/w NW and OB, (very small difference).
Grao-Cruces 2013	measured	ND	BIA	NS associations b/w MD adh. and BMI, % BF.
Grosso 2013	reported by adol.	IOTF	ND	Low adh. associated with OB.
Jennings 2011	measured	ND	BIA	Weight, BMI, BMI Z-score, WC, WHtR, and BF% were lower for children in the highest quintile of DQI score. MDS was not associated with weight status in these children.
Kontogianni 2008	reported by parents/ self reported (adol.)	IOTF	ND	NS associations b/w MD adh. and Leisure time, PA, BMI. ↓ass. b/w SB and KIDMED in children (Multple regression analysis).
Kontogianni 2010	reported by parents/ self reported (adol.)	IOTF	ND	KIDMED score was lower in OB adol. but not in OB child. ↓ass. b/w BMI and KIDMED score. ↓ass. b/w (high KIDMED+making breakfast+high meals freq.) and BMI. ↓ass. b/w BMI and ED, KIDMED score.
Lazarou 2009d	reported by parents	IOTF	ND	↓ass. b/w MD adh. and OB only in girls.
Lazarou 2010	reported by parents	IOTF	ND	High KIDMED CAT child. more likely to be NW. Child. with high KIDMED score were 83% less likely to be OW /OB.
Lydakis 2012	measured	IOTF	ND	Higher BMI and WC had significantly lower KIDMED score compared with lower BMI and WC.

Table 3.2: continued.

First Author/ Year	Anthropometry	OW/OB Definition	Body composition	Main results
Magriplis 2011	measured	IOTF	BIA	NS ass. b/w MD adh. and OW/OB, BMI and %BF.
Mazaraki 2011	measured	IOTF	ND	\uparrow ass. b/w BMI, WC, PA and KIDMED score.
McCourt 2014	measured	ND	ND	NS ass. b/w change in MDS and change in BMI
Monjardino 2014	measured	ND	ND	NS difference in BMI in the baseline evaluation and after 17 years.
Noale 2014	self reported	Cacciari	ND	NS ass. b/w MD adh. and PA, SB, BMI.
Ozen 2015	measured	WHO	ND	UW adol. who consumed Functional food had a higher risk to have low MD adh.; opposite trend among FF non-consumers.
Perez- Gallardo 2011	measured	ND	ND	↓ass. b/w MD adh. and BMI.
Roccaaldo 2014	measured	IOTF	ND	NS ass. b/w MD adh. and weight status.
Santomauro 2014	self reported	IOTF	ND	% of poor adh. was ↑ in UW students (35.9%). Multivariate logistic regression analysis showed that poor adh. was ↑ in students who played sports < "almost every day", who spent >3 h/d in SB. Being NW or OW/OB seem to be protective factors against poor adherence to MD.
Schröder 2010	measured		ND	WC, WHtR, BMI \downarrow with KIDMED quartiles. A 5-point \uparrow in KIDMED score was ass. with a mean \downarrow of 1.54 cm in WC.
Tognon 2014b	measured	IOTF	Tric and subsc ST	↓ass. b/w fMDS scores and OW/OB, WC, %BF. In the Prospective Intervention study high fMDS at baseline protected against increases in BMI, WC and WHtR.
Tsartsali 2009	measured	ND	ND	NS ass. b/w MD adh. BMI.

Abbreviations: NS = non-significant; MD = Mediterranean Diet; adh. = adherence; BP = blood pre sure; BMI = Body Mass Index; WC = waist Circumference; PA = physical activity; UW = underweight; NW = normal weight; OW = overweight; OB = obese/obesity; KIDMED CAT = MD adherence categories (Serra-Majem et al., 2004); BF = Body Fat; WHtR = waist to height ratio; DQI = Diet Quality Index; MDS = Mediterranean Diet Score (Trichopoulou et al., 1995); SB = Sedentary Behaviour; Child. = children; Adol. = adolescents; ED = Energy Density; NR = Not Reported; ND = Not Diagnosed; IOTF = International Obesity task Force; BIA = Bio Impedance Analysis; ST = Skinfold Thickness; fMDS = food frequency Mediterranean Diet Score (Tognon et al, 2014a).

Table 3.3: Association between adherence to the MD and lifestyle (physical activity an sedentary behavior) in 52 articles considered for the review (it continues in the next page).

First Author/ Year	Physical Activity	Sedentary Behavior
Arriscado 2014	↑ass bw MD adh. and PA, fitness. Higher MD adh was weakly associated with improved aerobic capacity and higher PA levels.	↓ ass bw MD adh. and ST. Higher MD adh was weakly associated with lower ST levels.
Arvaniti 2011b	NS ass bw MD adh. and PA.	
del Mar Bibiloni 2012a		\downarrow ass bw MD adh. and SB.
Farajian 2011	PA levels were higher in "average" MD adherers vs. "poor" MD adherers.	
Grao-Cruces 2013	"Good" MD adherers were more active than "poor" MD adherers.	"Good" MD adherers were less sedentary than "poor" MD adherers.
Grosso 2013	↑ass bw MD adh. and PA.	
Jennings 2011	68% children who practice ≥ 60 min/ MVPA/day had higher MDS than children who did not met the recommendation.	
Kontogianni 2008	LTPA and BMI did not differ among the 3 KIDMED CATs.	"Poor" MD adherers spent more time on SB than "average" or "good" MD adherers.
Lazarou 2009d		↓ ass. bw MD adh. and frequency and duration of TV, video and DVD viewing in boys.
Magriplis 2011	Children in "average" and "good" MD adh. had slightly higher PA levels	

First Author/ Year	Physical Activity	Sedentary Behavior
Martinez 2010		Sedentary and low active adol. showed lower MD adh. and lower risk of lower adh. than active and very active adolescents.
Mazaraki 2011	↑ass bw MD adh. and PA.	↓ ass. bw MD adh. and TV viewing.
Mc Court 2014	Participants with a high MDS had higher PA scores.	
Monjardino 2014	↑ass bw MD adh. and PA frequency in girls.	
Noale 2014	NS ass bw MD adh. and PA.	NS ass bw MD adh. and SB.
Ozen 2015	NS ass. bw high PA and low risk of MD adh. in FF consumers and non-consumers.	
Roccaldo 2014		↓ ass. bw MD adh. and ST (TVand PC); NS ass. bw MD adh. and having TV /PC in bedroom.
Santomauro 2014	↑ass bw "poor" MD adh. and non-physically active adol.	"Poor" MD adh. was higher in students who played sports < than "almost every day", spent >3 h/d in SB, used car/moped as main transport and were UW.
Schröder 2010	LTPA increased across KIDMED quartiles.	

Abbreviations: NS = non-significant; MD = Mediterranean Diet; adh. = adherence; BMI = Body Mass Index; PA = physical activity; UW = underweight; KIDMED CAT = MD adherence categories (Serra-Majem et al., 2004); MDS = Mediterranean Diet Score (Trichopoulou et al., 1995); SB = Sedentary behaviour; Child. = children; Adol. = adolescents; ST = Screen time; MVPA = Moderate-to-Vigorous-Physical-Activity; LTPA = leisure Time Physical Activity; FF = Functional Food. **Table 3.4**: Association between adherence to the MD and food groups/ nutrients/ number of meals in 52 articles considered in the review.

First Author/ year	Main results				
Arvaniti 2011a	↓ass. b/w MD adh. and salty snacks ↓ass. b/w MD adh. and likelihood to have asthma symptoms.				
Grosso 2013	↓ass. b/w KIDMED score and SFA, sweets, FF, cheeps, SD ↑ ass. b/w KIDMED score and carbohydrates, fibre, fruit, vegetables, pasta, fish, cheese.				
Jennings 2011	Energy intake \uparrow in quintile 5 than in quintile 1.				
Kontogianni 2008	↑ ass. b/w KIDMED score and calcium, vitamin C, fibre.				
Kontogianni 2010	↓ ass. B/w BMI and a pattern of higher eating freq.+breakfast consumption + higher KIDMED score.				
Lazarou 2009d	After adjustment for potential confounders: Children ≥average MD adh. were more likely to eat legumes, fish, seafood, nuts, bread, fruit, vegetables, olives, LGI foods, unrefined foods Children with good adh. consumed more fruit, traditional Cypriot foods.				
Magriplis 2011	Children with high sodium intake had ↑ MD adh.				
Martinez 2010	 ↑ ass. b/w high adh. and consumption of cereals, vegetables, fruit, legumes, fish, MUFA:SFA. ↓ ass. b/w high adh. and meat, whole milk, milk products, alcohol. 				
Monjardino 2014	\uparrow ass. b/w high adh. and calcium and energy intake.				
Noale 2014	Adolescents whose diet most closely adhered to MD assumed: higher median values of carbohydrates, fibre, vitamin B6, vitamin C, folic acid, vitamin A, vitamin D, iron, MUFA. Less median values of SFA and higher MUFA:SFA.				
Ozen 2015	\uparrow ass. b/w high adh. and functional foods.				
Schröder 2010	 ↑ ass. b/w KIDMED score and fruit, vegetables, cereals, fish, dairy products, proteins, fibre. ↓ass. b/w MD adh. and meat, pastry, sweets. 				
Schröder 2013	↓ass. b/w MD adh. and diets with high energy density.				
Serra-Majem 2003	↑ ass. b/w KIDMED score and fibre, calcium, iron, magnesium, potassium, phosphorus, all vitamins (except for vit E).				

Abbreviations: Ass.= association; b/w:=between; adh.= adherence; SFA=Saturated Fatty Acids; FF= Fast Foods; SD= soft Drinks; Freq.=frequency; vit. = vitamin; LGI= Low Glycaemic Index; MUFA= Mono Unsaturated Fatty Acids.

CHAPTER 4: MEDITERRANEAN DIET IN SCHOOL CHILDREN AND ADOLESCENTS IN THE CAMPANIA REGION

4.1 INTRODUCTION AND AIM

Unhealthy dietary habits of children are related with different cardiovascular risk factors, such as, obesity (Santos *et al.*, 2011), blood pressure (Niinikoski *et al.*, 2009), cholesterol (Royo-Bordonada *et al.*, 2006) and type-2 diabetes (Pereira *et al.*, 2005). In addition, unhealthy dietary habits come often along with other unhealthy behaviour such as sedentary ones.

The Mediterranean Diet (MD) is of special interest as it has been shown to have a positive impact on cardiovascular disease (Domínguez *et al.*, 2013) and metabolic syndrome (Kastorini *et al.*, 2011) even in children (del Mar Bibiloni *et al.*, 2011). The association with obesity, instead, is still unclear, both for adults (Buckland *et al.*, 2008) and for children (as we have shown in the systematic review we have presented in chapter 3).

It has to be noted that the Mediterranean countries are the ones shifting from the traditional MD model to a less healthy and more Western one. This is true especially for younger populations (Serra-Majem *et al.*, 2004; Tognon *et al.*, 2014b).

Promoting the MD could contribute to the increase in eating fruit and vegetables and the decrease of snack consumption, which are two critical points in the diet of children and adolescents. Because the habits of young people can be determined by individual (e.g. age, gender, food preferences, nutritional knowledge, attitudes), social (e.g. food pricing, education, family employment) and cultural factors (e.g. cultural trends, familial factors, peers

and product marketing) (Taylor *et al.*, 2005), it is important to identify the primary factors that influence diet in order to develop effective interventions.

4.2 METHODS

4.2.1 Study design and selection of participants

This study is a population-based cross-sectional nutritional and lifestyle survey carried out in the Campania Region during the academic years 2012-2013 and 2013-2014 among children and adolescents of secondary schools of Angri, a municipality of 32,000 inhabitants in Salerno province, equidistant (about 30kms) from the towns of Salerno and Naples and of S. Giorgio a Cremano, a municipality of 46,000 inhabitants on the outskirts of Naples.

Participation in the study was voluntary and the participants' parents gave written consent. Overall, 389 subjects from 10 to 14 years old were recruited.

4.2.2 Socio-demographic characteristics

Demographic and socioeconomic variables were assessed from a questionnaire including type of fathers' and mothers' job and their highest education level attained. The educational level was chosen as proxy of the socio-economic status (SES).

4.2.3 Anthropometric measurements

Anthropometric measures were taken from children wearing no shoes and light clothes. Weight was measured to the nearest 0.1 kg by means of a SECA 813 electronic scale. Height was measured to the nearest 0.1 cm with a SECA height rod. Circumferences were measured with a flexible steel tape (SECA) with 1 mm precision. Waist circumference (WC) was measured at the midpoint between the iliac crest and the lower coastal rib and recorded to the nearest 0.1 cm. Neck circumference (NC) was measured below the

cricoid cartilage, and afterwards, at the level of the mid cervical spine, as a measure of central obesity (Onat *et al.*, 2009). Measures of height and waist were taken three times and the mean value was considered for data analysis. WHtR, a marker of abdominal fat deposition independent of age (Ashwell *et al*, 2012), was calculated and the cut-point of ≥ 0.5 was used to identify adolescents with high abdominal adiposity (Ashwell and Hieh, 2005). Categories of normal weight (NW), overweight (OW) and obesity (OB) were defined according to Cacciari *et al.* cut offs for the Italian population (2006).

4.2.4 Dietary habits and adherence to the Mediterranean Diet

Usual dietary intake was assessed through a Food Frequency Questionnaire (FFQ) administered by interview by two trained nutritionists. The questions were read out by the interviewer who waited for the subjects to write down every answer whilst separated from each other. For each food item, respondents were asked to indicate the frequency of consumption per day, per week or per month. Never and seldom were also included and considered as 'zero'. All reported numbers were converted to daily frequency. The daily intake for each food group (fruit and vegetables; milk and yoghurt) was calculated by summing up the frequency of consumption of fruit plus vegetables or milk plus yoghurt. In the same way, the consumption (servings/day) of snack foods, soft drinks and the number of meals was also assessed.

MD adherence was assessed through the KIDMED questionnaire translated into Italian. The Italian version was previously trialed in a pilot study population of children and adolescents of 150 subjects.

4.2.5 Physical activity and sedentary behaviour

A questionnaire on lifestyle habits (physical activity and sedentary behaviour) was administered directly to the pupils. Questions on physical activity included type, frequency, duration of training sessions and level of different sports. Sedentary behaviour was assessed through questions on hours spent in watching television (TV), using computer (PC), playing videogames (VG) and using mobile phones when stationary. This last item has been included because cell phone has rapidly cemented its place as a media delivery platform for young people (Rideout *et al.*, 2010).

4.2.6 Statistical analysis

All statistical analyses were performed using the SPSS statistical software package version 19.0 (SPSS, Inc., Chicago, IL, USA). The level of significance was set at p<0.05. Variables were not normally distributed. Descriptive statistics, including medians, 25th and 75th percentiles, frequencies and percentages, were used to describe demographic and anthropometric characteristics of participants as well as healthy lifestyle behaviour. The non-parametric Mann–Whitney *U* test was used to compare differences in age, height, weight, BMI, WC and WHtR between genders. The Spearman's rank correlation test was performed to evaluate whether healthy lifestyle behaviour and junk snack food consumption correlated with each other. The χ^2 test was used to compare proportions for parental education levels or selected health behaviour between genders. Differences between means were tested using ANOVA.

4.3 RESULTS

4.3.1 General characteristics of the population studied

The total number of participants was 389 (193 males and 196 females), with a participation rate of 95.6%. The age ranged between 10.2 and 14.9 years; mean age 12.2 ± 1.0 years.

General and anthropometric characteristics are shown in Table 4.1: mean BMI was 22.0 ± 4.4 kg/m² (range: 12.0-36.0 kg/m²); mean BMI z-score was 0.3 ± 1.1 (range: -3.43-3.06); mean WC was 71.45 ± 10.0 cm (range: 48.0-113.0 cm); mean WHtR was 0.46 ± 0.06 (range: 0.35-0.63); mean NC was 31.25 ± 2.7 cm (range: 23,0-40,0 cm). Males were taller and had larger NC than girls (p=0,001).

Weight status was distributed as follows: 62.5% of the studied population was NW, 19.3% OW and 18.3% was OB, with no difference between genders (Figure. 4.1a and Figure. 4.1b).

SES was determined by the parents' educational level, as shown in Figure 4.2a and Figure 4.2b. The educational level was homogenously distributed between genders as follows: for the fathers 6.9% attended only elementary school, 39.8% attended middle school, 42.9% had a high school diploma and 9.5% had a graduate/postgraduate degree. For the mothers 9.3% attended elementary school, 40.6% also attended middle school, 41.9% had a high school diploma and 7.7% had a graduate/postgraduate degree.

Lifestyle characteristics are shown in Table 4.2. Regarding sedentary behaviour boys had a median value of 2 hours/day watching TV (IQR: 1-3), of 1 hour/day spent on a PC (IQR: 0-2), of 1 hour/day playing videogames

(IQR: 0-2), of 1 hour/day using the mobile phone when stationary (IQR: 0-2). Finally, boys spent a median of 5 hours/day in total sedentary behaviour (IQR: 4-8). Girls had a median value of 2 hours/day watching TV (IQR: 1-3), of 1 hour/day spent on a PC (IQR: 0-2), of 0 hour/day playing videogames (IQR: 0-1), of 1 hour/day using the mobile phone when stationary (IQR: 0-3). Finally, girls spent a median of 5.5 hours/day in total sedentary behaviour (IQR: 3-9).

A significant difference between boys and girls was observed in total screen time (TV+PC+VG) (p=0.003): 15.6% of the boys spent 1-2 hours/day on a screen, 34.7% spent 3-4 hours/day, 25.5% spent 5-6 hours/day, 8.3% spent 7-8 hours/day, 6.7% spent 9-10 hours/day and 9.2% spent more than 10 hours/day on a screen, 24.5% of the girls spent 1-2 hours/day on a screen, 32.2% spent 3-4 hours/day, 20.4% spent 5-6 hours/day, 10.7% spent 7-8 hours/day, 6.7% spent 9-10 hours/day and 3% spent more than 10 hours/day on a screen (Figure 4.3).

4.3.2 Eating patterns

Analysing the nutritional habits of the adolescents (Table 4.3), girls consumed vegetables, vegetables and fruit, yoghurt more frequently than boys (p=0.002; p=0.048; p=0.003 respectively), who, instead, consumed more frequently milk and soft drinks (p=0.028 and p=0.031 respectively). Regarding the median weekly consumption of the main recommended and not-recommended foods, girls consumed vegetables, fruit and vegetables and yoghurt more frequently than boys (p=0.002; p=0.048; p=0.003 respectively), who, instead, consumed milk and soft drinks more frequently than girls (p=0.028 and p=0.031 respectively).

Analysing the correlations between the different food groups, adolescents who ate vegetables regularly were also in the habit of eating fish and yoghurt (p<0.001). The contrary can be said for soft drinks (p<0.001) and snacks (p=0.002). Moreover, soft drinks consumption was positively correlated with snack consumption (p<0.001) and negatively correlated with fish consumption (p=0.018).

Analysing the possible predictive factors of eating patterns, a linear generalised model was constructed (Table 4.4). Girls consumed vegetables (p=0.001), fruit and vegetables (p=0.044) and yoghurt (p=0.003) more frequently than boys, who, instead, consumed more frequently milk (p=0.032) and soft drinks (p=0.033).

The consumption of vegetables, fruit and fruit and vegetables decreased with age (p=0.008; 0.034; 0.005 respectively), while snack consumption and soft drink consumption increased with age (p=0.022 and p=0.049 respectively).

The fathers' educational level was positively associated with the frequency of consumption of fruit (p=0.033) and fish (p=0.041), while the mothers' educational level was negatively associated with snack consumption (p=0.030).

The number of meals/day was negatively correlated with the consumption of fruit (p=0.016); fruit and vegetables (p=0.043) and positively correlated with the consumption of milk (p=0.006) and soft drinks (p=0.002). Finally, skipping breakfast was obviously directly associated with the consumption of milk (p=0.029) and milk and yoghurt (p=0.012).

Eating patterns and sedentary behaviour

Eating patterns were correlated with sedentary behaviour and physical activity, after adjustment for age and gender. As we can observe from Table 4.5, physical activity was not correlated with any of the nutritional habits, while total screen time and screen time + mobile phone use correlated with

vegetable consumption (p<0.01). None of the components of sedentary behaviour correlated with either the number of meals, or milk or milk + yoghurt consumption or snack consumption; while all components of sedentary behaviour positively correlated with soft drink consumption (p<0.001).

Eating patterns and adiposity

Finally, eating patterns were correlated with adiposity indexes (Table 4.6). After adjustment for gender and age there was an interesting negative association between snack consumption and 3 out of 4 adiposity indexes (WC, p=0.012; WHtR, p=0.003; NC, p=0.025). Moreover, the frequency of meals/day was negatively associated with BMI z-score (p=0.012). The frequency of consumption of all other food groups (fruit, vegetables, fish, milk, yogurt and soft drinks) was not significantly correlated with the adiposity indexes.

4.3.3 Adherence to the Mediterranean Diet

The adherence to the MD was analysed through the KIDMED index.

The sample was divided into 3 categories (poor, average and good), as suggested by Serra-Majem *et al.* (2004). 23,9% of the sample poorly adheres to the MD, 61.4% showed an average level of adherence, while 14.7% presented good adherence to the MD. There was no difference between genders (Figure. 4.4 a-b).

As shown in Table 4.7 the KIDMED score mean was 5.2 ± 2.3 with no difference between genders. About 52.4% of the population consumed 1 portion of fruit/day, 35.2% also consumed a second fruit/day; 49.4% consumed 1 portion of vegetables /day while 26.0% also consumed a second portion of vegetables /day; 63.2% consumed fish more than twice/week; 17.0% went to a fast food restaurant more than once/week; 80.7% ate pulses

more than once/week; 94.3% consumed pasta almost every day; 49.9% consumed cereals or grains for breakfast; 21.1% consumed nuts more than twice/week; 93.3% used olive oil as the main condiment; 49.4% skipped breakfast; 78.4% had a diary product for breakfast; 61.1% consumed commercial baked goods or pastries for breakfast; 50.1% ate 2 yoghurts and/or 40g of cheese every day; 49.6% consumed cakes and/or sweets several times/day. Girls consumed more vegetables than boys (p =0.035), while boys went to fast food restaurants more often (p=0.012) and consumed more commercial baked goods and pastries for breakfast (p=0.004).

Analysis of the different KIDMED catergories

Interestingly, analysing the subjects with a poor adherence to the MD and those with a good adherence (Table 4.8), it can be observed that 30.8% of the studied population classified as good-adherers did not consume either fruit or vegetables as internationally recommended, 40.8% skipped breakfast and 46.9% had commercial baked goods or pastries for breakfast.

Analysing the consumption of different food groups (times/week) and the number of meals/day between the lowest and the highest category of KIDMED score (Table 4.9), there was a significant difference in the consumption of fruit (p<0.001), vegetables (p<0.001), pulses (p<0.001), fruit and vegetables (p<0.001), fish (p<0.001), soft drinks (p<0.001) and snacks (p=0.002); while no difference was found in the consumption of milk (p=0.371), yoghurt (p=0.141) and milk and yoghurt (p=0.139). The frequency of meals was also directly associated to the category of KIDMED score (p<0.001).

Adherence to the Mediterranean Diet and age

As we can obserbe in Figure 4.5, the KIDMED score tended to decrease with age. In fact, the KIDMED mean score is 5.53 ± 2.21 at 11 years old, 5.09 ± 2.16 at 12 years old and 4.59 ± 2.57 at 13 years old (p=0.07). Analysing

all "yes" answers of the KIDMED questionnaire (Figure 4.6), we can observe that the consumption of 1 portion of fruit/day significantly decreased with age (p=0.027) and the consumption of several cakes and sweets/day significantly increased with age (p=0.031).

Adherence to the Mediterranean Diet, physical activity and sedentary behaviour

Analysing the correlations between KIDMED score and both sedentary behaviour and physical activity, we found, after adjustment for age and gender, a signidificant correlation between KIDMED score and time spent on PC (p=0.001), VG (p=0.014), mobile phone (p<0.001) and total screen time (p<0.001). Consistent results were obtained when making the same correlations through the KIDMED categories.

Adherence to the Mediterranean Diet and adiposity indexes

Some associations where found when adiposity indexes (BMI z-score, WC, WHtR and NC) were correlated with the 16 components of KIDMED index: WC was positively associated to the consumption of fish > 2/week (p=0.009) and to the frequent consumption of sweets and cakes (p=0.023). Moreover, WHtR was associated to the frequent consumption of sweets and cakes (p=0.015).

4.4 DISCUSSION

Almost 20% of the school children and adolescents of the present study are overweight and more than 15% are obese. These results are slightly different from what reported in our region from HBSC (Mazzarella and Pizzuti, 2011) if we consider overweight and obese separately, but they become consistent if we cluster overweight and obese together. This can be also due to the fact that HBSC data are referred and not measured, and, as Mazzarella *et al.* (2011) show in their report there is a under self- perception of the weight status of adolescents.

Regarding sedentary behavior, the subjects of the studied population appear slightly more sedentary than their peer in the Campania region (Mazzarella and Pizzuti, 2011), although the most recent data collection for this population date back to 2009 and the literature shows how rapidly this particular component of the lifestyle of children and adolescents changes. As an example we can think about the ownership increase of mobile phones among children and adolescents, which was more than 1,5 times between 2004 and 2009 in the U.S.A. (Rideout *et al.*, 2010). The gender differences, instead, are consisted with the regional data of HBSC.

4.4.1 Eating patterns

The consumption of fruit and vegetables is commonly promoted because they are nutrient-dense foods (vitamins, minerals, fibre and water) with a potential effect, even if relatively small, in protecting against adiposity in children (Davis *et al.*, 2007). In agreement with previous surveys (Mazzarella and Pizzuti, 2011; Larson *et al.*, 2007) the average consumption of fruit and vegetables was very low in the studied population, with only a low percentage of them (23.2%) consuming at least 5 servings/day. In accordance to HBSC regional data, 55% of the adolescents consumed fruit less than 1 time/day and 61.7% consumed vegetables less than 1 time/day. Moreover, the shown differences of fruit and vegetable consumption among genders are very often observed in the literature and preference appears as the strongest mediator of the difference in fruit and vegetable intakes (Bere *et al.*, 2008).

Another marker of a healthy diet is eating non-energy-dense dairy products (ADA, 2007), which are an important source of calcium (Ross, 2011). In our sample only 2.3% of the adolescents met the guidelines for milk and yoghurt consumption, in line with previous data regarding our region (Iaccarino Idelson *et al.*, 2014).

A marker of unhealthy eating patterns is the consumption of soft drinks. The present data are perfectly in line with HBSC ones (only 9% of the studied adolescents do not consume soft drinks at all, and 9% consumed them almost every day).

Daily snack consumption was found in 55% of the studied population. If we compare this datum with the one obtained in the previous study (Iaccarino Idelson *et al.*, 2014) on older adolescents (15-17 years old), we can observe an increase of snack consumption with age in Campania Region, which is in line with other studies and surveillance systems (Mazzarella and Pizzuti, 2011; Currie *et al.*, 2012). Both the European surveillance system (Currie *et al.*, 2012) and the regional one (Mazzarella and Pizzuti, 2011) show how eating patterns as a whole worsen as a child becomes adolescent; the subjects studied in the present study significantly decreased their consumption of fruit and vegetables and increased the consumption of snacks and soft drinks from the first to the last year of middle school (11-13 years old).

Apparently, parental educational level affected good and bad eating habits (Béghin *et al.*, 2014). In the present study, the fathers' educational level was positively associated with the frequency of consumption of fruit and vegetables and fish, while the mothers' educational level was negatively associated with snack consumption.

Sedentary behaviour is linked with dietary behaviour, and this also possibly explains the link between sedentary behavior and OW/OB (Robinson, 2001). Sedentary behaviour such as TV viewing is believed to be associated with dietary behaviour through several mechanisms. One of these is the influence of food advertisements on food choice and intake (Boyland *et al.*, 2011; Harris *et al.*, 2009; Robinson, 2001). The consumption of highly advertised
foods might in turn lead to the replacement of less advertised foods such as fruit and vegetables. Sedentary behaviour, such as TV, computer or videogame use are also found to serve as distractors. This disruption of habituation to food cues leads to overeating and thereby to an increase in energy intake (Epstein *et al.*, 2009; Temple *et al.*, 2007). A recent systematic review highlighted the existence of consistent associations between sedentary behaviour and unfavorable dietary behaviour in all age groups (Pearson and Biddle, 2011).

In our study, eating patterns were correlated with sedentary behaviour and physical activity. All the components of sedentary behaviour negatively correlated with vegetable consumption (p<0.01), vegetable and fruit consumption (p<0.03) and positively correlated with soft drinks consumption (p<0.001). Moreover, total screen time was associated to the consumption of snacks (p=0.005).

Given the growing contribution of snacks to dietary intake and the need for effective strategies to reduce obesity, it is important to consider whether snacking behaviour contribute to high BMI in childhood.

Recent analyses of trends in dietary intake have shown there were significant increases in the contribution of energy-dense, nutrient-poor foods to snacking kilocalories over the past few decades (Larson *et al.*, 2013). The majority of studies either found no evidence of a relationship between snacking behaviour and weight status or found evidence indicating that young people who consumed more snacks were less likely to be obese; the negative association between snack consumption and the adiposity indexes (WC, p=0.012; WHtR, p=0.003; NC, p=0.025) found in the present study is a further contribution to this hypothesis.

4.4.2 Adherence to the Mediterranean Diet

The adherence to the MD was analysed through the KIDMED index.

The sample was divided into 3 categories (poor, average and good), as suggested by Serra-Majem *et al.* (2004). As we can see from the systematic review presented in chapter 3, our data are consistent with the other studies which evaluated the adherence to MD through the KIDMED score and its categories. In fact, 23.9% of the adolescents analysed in this study poorly adhered to the MD (compared to the mean of 25%), 61.4% showed an average level of adherence (compared to the mean of 52%), while 14.7% presented good MD adherence (compared to the mean of 21%).

As far as is known, there are only 2 other studies presenting data on Southern Italian populations: Roccaldo *et al.* (2014) on children and Grosso *et al.* (2013) on adolescents. Comparing our data with Grosso *et al.* (2013) we found a strong similarity, possibly hypothesizing that adolescents of Southern Italy adhere to the MD in the same way.

The analysis of each answer of the KIDMED questionnaire was done in the present study, as in other 13 ones. Only this study, in addition to Roccaldo *et al.* (2014), evaluated the geographical differences for each food item. Although the KIDMED score is intended to be used from all the populations traditionally adopting the MD, cultural national differences can be noted. Eating pasta and/or rice almost every day is an Italian characteristics (as shown in Roccaldo's and the present study the consumption of pasta/rice is > than 90%, or about 85% in Santomauro *et al.* (2014) but not Spanish (i.e. in the populations analysed by Arriscado *et al.* (2014) and by Ayechu and Durà (2010) the regular consumption of pasta/rice is even less frequent (from 18.3% in

Grigoropoulou's urban population (2013) to 36.4% in Bargiota's data (2013)). We can observe differences also regarding both the consumption of dairy products for breakfast and of nuts, but the real universal ingredient of the MD is olive oil used as main condiment. In fact, its regular use and consumption in most of the studied population>90%, but in any case < 73%.

During the shift from childhood to adolescents the quality of the diet tends to decrease (Currie *et al.*, 2012); so apparently does adherence to the MD. Our data support this hypothesis, as did other 7 studies among the ones which have analysed this relationship. The others 14 did not found any significant correlation between adherence to the MD and age, while no one found an opposite association. In particular, we observed a decrease in the consumption of fruit, but an increase in the consumption of cakes and sweets, in conformity with regional data on the nutritional habits of adolescents (Mazzarella and Pizzuti, 2011).

Interestingly, the analysis of the different categories of KIDMED in relation food groups was made only in the present study and by Farajan *et al.* (2011). Both the present study and Farajan's one found significant differences in the consumption of fruit, vegetables, pulses, fish, soft drinks and snacks, but the population studied by Farajan *et al.* (2011) had better scores for all items. This can be due to the difference in sample size. We observed, in fact, that 30.8% of the studied population classified as good-adherers did not consume fruit and vegetables as internationally recommended, 40.8% skipped breakfast and 46.9% had commercial baked goods or pastries for breakfast.

As we have observed in the systematic review (chapter 3) 47.8% of the studies reported no significant association between adherence to MD and BMI; our study could be included in this group. Indeed, as far as is known, this is the first study which analysed the relationship between the 16 items of

KIDMED questionnaire and adiposity indexes. CV and WHtR were significantly higher in the adolescents who gave a "yes" answer to the question on sweets and cakes (p=0.023; p=0.015 respectively), while were lower in the adolescents who gave a "yes" answer to the question on fish (p=0.009).

Analysing the correlations between KIDMED score and both sedentary behaviour and physical activity, we found, after adjustment for age and gender, a significant negative correlation between KIDMED score and time spent on PC (p=0.001), VG (p=0.014), mobile phone (p<0.001) and total screen time (p<0.001).

4.5 CONCLUSIONS

The results of this study indicate that adolescents' eating patterns and lifestyle habits have to be improved. It is very important to analyse the factors influencing eating patterns and lifestyle of children and adolescents in the Campania region, because it is the Italian region with the highest overweight and obesity rate. Our contribution in analysing the single components of KIDMED in relationship with adiposity and the analysis of the relationship between the KIDMED categories and the consumption of the main food groups is a tentative to go in more depth of this public health concern.

The results of the present study suggest that strategies have to take into account the food culture of target populations and that the decrease of sedentary behavior has to be the main goal, as all the components of this behaviour are associated with the adherence to the Mediterranean Diet.

	Total (n 389)	Boys (n 193)	Girls (n 196)	p value
Age (years)	11.7 ± 1.0	11.7 ± 1.0	11.8 ± 1.0	0.7
Weight (kg)	54.2 ± 13.5	54.8 ± 14.0	53.6 ± 13.1	0.2
Height (cm)	154.9 ± 8.7	155.8 ± 9.7	153.9 ± 7.5	0.001
BMI (kg/m ²)	22.0 ± 4.4	21.9 ± 4.5	22.1 ± 4.4	0.5
MI z-score	0.3 ± 1.1	0.4 ± 1.0	0.3 ± 1.2	0.5
WC (cm)	71.45 ± 10.0	73.4 ± 10.1	69.5 ± 9.4	0.1
WHtR	0.46 ± 0.06	0.47 ± 0.06	0.45 ± 0.06	0.2
NC (cm)	31.25 ± 2.7	32.3 ± 2.7	30.25 ± 2.3	0.001

Table 4.1: General and anthropometric characteristics of the 389 adolescents, by gender.



adolescents (Cacciari et al., 2006).



Figure 4.1b: Weight status for 389 females adolescents (Cacciari *et al.*, 2006).



p = NS



Figure. 4.2a: Fathers' educational level of the 389 adolescents.



Figure. 4.2b: Mothers' educational level of the 389 adolescents.

Maximum educational level attained: Elementary school: 9.3%; middle school: 40.6%; high school: 41.9%; graduate/post graduate diploma: 7.7%.

Maximum educational level attained: Elementary school: 6.9%; middle school: 39.8%; high school: 42.9%; graduate/post graduate diploma: 9.5%.

	Boys (N	l = 193)	Girls (N	N = 196)	p value
	Median	(IQR)	Median	IQR	
Sport (h/week)	3	0-5	2	0-4	<0.001
TV (h/day)	2	1-3	2	2-3	0.763
PC (h/day)	1	0-2	1	0-2	0.283
Videogames (h/day)	1	0-2	0	0-1	<0.001
Mobile phone (h/day)	1	0-2	1	0-3	<0.001
Screen time (h/day)	4	3-6	4	2-6	0.003
Screen time + mobile phone (h/day)	5	4-8	5.5	3-9	0.592

Table 4.2: Lifestyle characteristics of the studied population (389 adolescents).

IQR = 25th - 75th percentiles.



Figure 4.3: Total screen time (TV + PC + VG) in the studied population (389 adolescents).

p=0.003.

	Total (1	n 389)	Boys (1	Boys (n 193)		Girls (n 196)	
	Median	IQR	Median	IQR	Median	IQR	
Vegetables/ week	5	3-7	5	3-7	6	3-10	0.002
Fruit/week	7	3-10	6	3-9	7	3-11	0.932
Pulses/week	3	2-4	3	2-4	3	2-4	0.293
Fruit and veg/ week	15	10-22	15	10-20	16	11-23	0.048
Fish/week	2	1-2	2	1-2	2	1-2	0.417
Milk/week	7	3-7	7	4-7	7	2-7	0.028
Yoghurt/ week	1	0-2	0	0-2	1	0-3	0.003
Milk and yogurt/week	7	5-9	7	5-9	7	4-9	0.606
Snacks/week	7	4-10	7	4-10	7	4-10	0.164
Soft drinks/ week	3	1-5	3	2-5	2	1-4	0.031
N. meals/day	4	4-5	4	4-5	4	4-5	0.238

Table 4.3: Eating patterns of the studied population (389 adolescents).

IQR = 25th - 75th percentiles.

	Gender	Age	Fathers' educational level	Mathers' educational level	Meals/day	Skip breakfast
Vegetables/ week	↑ (p=0.001)	↓ (p=0.008)				
Fruit/ week		↓ (p=0.034)	↑ (p=0.033)		↓ (p=0.016)	
Fruit and vegetables/ week	↑ (p=0.044)	↓ (p=0.005)			↓ (p=0.043)	
Fish/week			(p=0.041)			
Milk/week	↓ (p=0.032)				↑ (p=0.006)	↑ (p=0.029)
Yoghurt/ week	↑ (p=0.003)					
Milk and yoghurt/ week						↑ (p=0.012)
Snacks/ week		↑ (p=0.022)				
Soft drinks/ week	↓ (p=0.033)	(p=0.049)		↓ (p=0.030)	(p=0.002)	

Table 4.4: Possible predictive factors of nutritional habits (generalised linear model) in the studied population (389 adolescents).

 \uparrow = positive significant correlation; \downarrow = negative significant correlation

Table 4.5: Correlation between nutritional habits and sedentary behaviour and physical activity after adjustment for age and gender in the 389 adolescents of the sample studied.

	Meals/day	Fruit/wk	Veg/wk	Fruit and veg/wk	Fish/wk	Milk/day	Yoghurt/day	Milk and yoghurt/day	Snacks/wk	Soft drinks/wk
TV(h/day)			↓ (0=0.025)							↑ (p<0.001)
PC (h/day)					↑ (p=0.018)					↑ (p<0.001)
Videogames (h/day)										↑ (p=0.001)
Mobile phone (h/day)										↑ (p<0.001)
Screen time (h/day)			↓ (p=0.024)							↑ (p<0.001)
Screen time + mobile phone (h/day)			↓ (p=0.024)							↑ (p<0.001)
Sport (h/week)										

 \uparrow = positive significant correlation; \downarrow = negative significant correlation

Table 4.6: Association between adiposity indexes and eating patterns in the 389 adolescents studied, after adjustment for age and gender.

	Meals/day	Fruit/wk	Veg/ wk	Fruit and veg/Wk	Fish/wk	Milk/day	Yoghurt/day	Milk and yog/day	Snacks/Wk	Soft drinks/Wk
BMI z-score	↓ (p=0.012)									
WC (cm)									↓ (p=0.012)	
WHtR									↓ (p=0.003)	
NC (cm)									↓ (p=0.025)	

WC: waist circumference; WHtR: waist to height ratio; NC: neck circumference; Veg.: vegetables; Yog.: yoghurt.

Figure. 4.4 a-b: Adherence to the MD through the identification of the 3 categories (as suggested by Serra-Majem, *et al.*, 2004) in the 389 studied adolescents (p = 0.111).



a.



b.

"Poor" category: KIDMED score <4

"Average" category: KIDMED score 4-7

"Good" category: KIDMED score >7

	Total (n 389)	Boys (n 193)	Girls (n 196)	р
Takes a fruit every day	52.4	51.8	53.1	0.805
Has a second fruit every day	35.2	35.2	35.2	0.995
Has fresh or cooked vegetables once a day	49.4	44.6	54.1	0.060
Has fresh or cooked vegetables > 1/ day	26.0	21.2	30.6	0.035
Consumes fish regularly (> twice/week)	63.2	62.2	64.3	0.666
Goes > 1/ week to a fast food restaurant	17.0	21.8	12.2	0.012
Eat pulses >1/ week	80.7	77.2	84.2	0.081
Consumes pasta or rice≥5 times/week	94.3	95.9	92.9	0.201
Has cereals or grain for breakfast	49.9	52.3	47.4	0.336
Consumes nuts regularly (> twice/week)	21.1	23.8	18.4	0.186
Uses olive oil at home	93.3	91.7	94.9	0.208
Skips breakfast	49.4	47.2	51.5	0.388
Has a dairy product for breakfast	78.4	81.9	75.0	0.100
Has commercial baked goods for breakfast	61.1	68.2	54.1	0.004
Takes 2 yoghurts and/or 40g of cheese daily	50.1	46.6	53.6	0.171
Takes sweets/candies several times a day	49.6	49.7	49.5	0.960
KIDMED score (mean ± standard deviation)	5.2 ± 2.3	5.0±2.3	5.3±2.3	0.570

Table 4.7: Percentage of the "yes" answers to the single components of the KIDMED index and KIDMED score mean, by gender in the 389 studied adolescents.

	"yes"		
Questions	KIDMED score <4	KIDMED score ≥8	р
Takes a fruit everyday	18.3	89.5	< 0.001
Has a second fruit everyday	17.2	68.4	<0.001
Has fresh or cooked vegeTableles 1/day	17,2	84.2	< 0.001
Has fresh or cooked vegeTableles > 1/ day	6.5	68.4	< 0.001
Consumes fish regularly (> 2/week)	47.4	52.6	<0.001
Goes > 1/ week to a fast food restaurant	94.4	5.6	<0.001
Eat pulses >1/ week	59.1	96.5	<0.001
Consumes pasta or rice ≥5 times/week	89.2	100.0	0.010
Has cereals or grain for breakfast	23.7	84.2	<0.001
Consumes nuts regularly (> 2/week)	11.8	40.4	<0.001
Uses olive oil at home	86.0	100.0	0.003
Skips breakfast	73.1	38.6	<0.001
Has a diary product for breakfast	58.1	93.0	<0.001
Has commercial baked goods for breakfast	73.1	50.9	<0.001
Takes 2 yogurt and/or 40g of cheese daily	25.8	82.5	< 0.001
Takes sweets/candies several times / day	69.9	17.5	<0.001

Table 4.8: Differences in the "yes" answers of the 389 studied adolescents

 between "poor adherers" and "good adherers".

Table 4.9 :	Association	between	food	groups	and	categories	of	KIDMED
score in the	389 studied	adolescen	ts.					

	"Poor" ad KIDMED s	herence score < 4	"Good" : KIDMEI	р	
	Median	IQR	Median	IQR	
Number meals/die	4	3-5	5	4-5	< 0.001
Fruit/week	4	2-7	7	7-14	< 0.001
Vegetables/week	3	2-5	10	6-14	<0.001
Pulses/week	3	1-3	3	2-4	<0.001
Fruit and veg/week	11	6-15	24	18-29	<0.001
Fish/week	1	1-2	2	1-3	< 0.001
Milk/week	7	2-7	7	4-7	0.371
Yogurt/week	0	0-2	1	0-3	0.141
Milk + yogurt/week	7	3-9	7	6-9	0.139
Snacks/week	7	5-13	5	3-7	0.002
Soft drinks/week	3	2-7	2	1-3	< 0.001

IQR: 25th-75th percentile.



Fig 4.5: KIDMED mean score (± standard deviation) *vs.* age in the studied population (389 adolescents).

Fig. 4.6: Percentage of "yes" answers of the KIDMED questionnaire in the 389 studied adolescents according to age.



The "yes" answer to the first question: **Takes a fruit everyday** decreased with age (p=0.027). The "yes" answer to the last question: **Takes sweets/candies several times a day** increased with age (p=0.031).

CHAPTER 5: CONCLUSIONS

It is known that unhealthy eating patterns and sedentary behaviour are linked with the onset of overweight and obesity and that obese children will probably become obese adults, with a shorter life expectancy and higher risk of contracting cardio-vascular diseases and cancer.

Moreover, childhood and adolescence are crucial periods of life when taste, food habits and lifestyle attitudes become established. This means that if young individuals learn to eat in a healthy way, and to be physically active, they will probably preserve these habits and their health in adulthood.

The Campania region has the highest rate of childhood overweight and obesity in Italy, which is in turn one of the European countries with the highest rate of overweight and obesity in children and adolescents. Possible causes, apart from the dis-equilibrium between energy intake and calory expenditure, are social and environmental factors, such as numbers of school refectories, parental socio-economic status, barriers to physical activity and more sedentary behaviour, such as having TVs in children's bedrooms.

Therefore, urgent need for effective strategies is required at all levels (educational, medical, legal and political) in order to face the multiple risks linked to unbalanced diet and sedentary lifestyle of children.

The principal aim of this thesis has been the analysis of eating patterns and lifestyle of children and adolescents in Campania, in order to assess the association with different possible predictive factors such as age, gender and socio-economic status and with adiposity indexes. Moreover, after the results of the systematic review on the adherence to the Mediterranean Diet in children and adolescents presented in this thesis, the association between adherence to the Mediterranean Diet and possible predictive factors and with adiposity indexes has been analysed in a sample of adolescents in the Campania region.

Only by reaching a deep understanding of the causes and possible associations between childhood eating patterns and lifestyle with environmental factors, effective strategies can be created and implemented in order to decrease the enormous prevalence of childhood obesity in Campania and all its negative consequences.

More specifically, the first study presented in this thesis indicates that adolescents do not often meet health recommendations on diet and physical activity, In particular, watching TV for ≥ 2 h/day is the marker more strictly related to abdominal fat in adolescence. It is suggested that the calculation of WHtR should be included in health surveillance systems, because not only is it a predictor of cardiovascular risk but also it is an indicator of unhealthy lifestyles.

Adherence to a Mediterranean Diet can significantly decrease the risk of overall mortality. These results are also strictly concordant with current guidelines and recommendations from all the major scientific associations that strongly encourage a Mediterranean-like dietary pattern for primary and secondary prevention of major chronic diseases.

The systematic review presented in this thesis has aimed to analyse the level of adherence to the MD of children and adolescents and its possible predictive factors, its association with adiposity indexes, lifestyle and body composition variables and its nutritional adequacy. As overweight children are likely to become obese adults, and healthy eating patterns, as well as lifestyle attitudes learnt during childhood and adolescence become established in adulthood, it has been important to understand if the Mediterranean Diet could be considered a useful tool for the promotion of a healthy diet.

Fifty two articles have been systematically reviewed for this purpose and it has been concluded that it is important to promote the Mediterranean Diet especially in low socio-economic families and to create "smart" and effective strategies specific to adolescent groups, who are at major risk, because the adherence to the Mediterranean Diet declines with age.

The association between adherence to the Mediterranean Diet and higher physical activity levels and lower sedentary behaviour reflects a virtuous and positive cycle that some children and adolescents undertake. This result gives a direction as to where to put effort into the development of prevention strategies.

It is important to promote the Mediterranean Diet among children and adolescents because it has emerged from the analysis of the reviewed articles that MD adherence is associated with higher intake of fibre, calcium, iron, vitamin C, better MUFA:SFA and lower intake of SFA, which are critical nutrients during adolescence. Moreover, high adherence to the Mediterranean Diet is positively associated with the intake of the most recommended foods for health (fruit and vegetables, legumes, fish, whole grain cereals) and negatively associated with soft drinks, snacks, fast foods and sweets, whose consumption is often common among children and adolescents.

Analyzing the level of adherence to the Mediterranean Diet in a sample of adolescents from the Campania region (the third study), it has been found that this population did not differ from the others in Italy and Europe. The analysis of the different categories of KIDMED in relation to food groups found significant differences in higher consumption of fruit, vegetables, pulses, fish, and lower consumption of soft drinks and snacks with better KIDMED scores, although unhealthy eating patterns (such as skipping breakfast, not consuming enough fruit and vegetables, or consuming too many sweets and cakes) also remained in the "good-adherer" category.

As far as is known, this is the first study which analysed the relationship between the 16 items of the KIDMED questionnaire and the adiposity indexes and it was found that CV and WHtR were significantly higher in the adolescents who stated that they consumed several sweets and cakes during the day. This conclusion suggests that the KIDMED index can be applied to better understand eating patterns of children and adolescents and that it gives specific indications of what food or food group has to be encouraged or discouraged.

Finally, all the components of sedentary behaviour (TV viewing, videogame playing, time spent on PC) were associated with poor adherence to the Mediterranean Diet, suggesting that strategies are needed which strongly target the decrease of all sedentary beahviour.

Although further research is needed, and researchers, scientific associations, governmental or intergovernmental bodies are far from finding solutions which would allow children and adolescents to live as long as their parents, this study is the attempt to put another tile in this complicated mosaic.

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