CONSUMPTION OF A MEDITERRANEAN-LIKE DIET AND ITS RELATIONSHIP WITH GROWTH, BODY FATNESS, AND PUBERTY

by

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ABSTRACT OF THE DISSERTATION

Consumption of a Mediterranean-like Diet and its Relationship with Growth, Body Fatness, and Puberty

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In recent years, there has been a population-level trend toward earlier puberty, which presents psychological and clinical risks to girls. Body fatness and earlier puberty are also risk factors for problems including adult obesity and breast cancer. Diet is a primary and modifiable factor that can influence puberty and growth. Current research on a Mediterranean-like dietary pattern in regard to these outcomes is limited; no studies have examined its role in puberty outcomes or longitudinal growth, and only one study has examined a Mediterranean-like diet (MD) and body mass index (BMI) in a healthy U.S. pediatric population. We aimed to address these gaps in the literature by evaluating the role of MD adherence in puberty, growth, and body fatness in *The Jersey Girl Study*. We developed an index for assessing adherence to a Mediterranean-like diet and evaluated its relationship with cross-sectional and longitudinal outcomes. Our sample was a cohort of 202 girls who resided in New Jersey and were 9 or 10 years old at baseline. Data were collected from a baseline study visit and physical examination, a 3-day dietary recall, a general baseline questionnaire filled out by girls' mothers, and annual follow-up questionnaires on growth and puberty outcomes.

Multivariable Poisson regression models showed that high adherence (score 6-9) was significantly associated with lower prevalence of thelarche at baseline compared to girls with low

adherence (score 0-3) (Prevalence Ratio: 0.65, 95% confidence interval (CI): 0.48-0.90 in the fully adjusted model). Further analysis suggested that this may have been driven by consumption of fish and reduced/low/non-fat dairy. Multivariable linear regression models to examine mean age at thelarche also suggested a nonsignificant trend of later age at thelarche with higher MD adherence. Multivariable Cox proportional hazards models found that girls with higher MD adherence had significantly longer time to menarche (Hazard Ratio: 0.45, 95% CI: 0.28-0.71 in the fully adjusted model for girls with high vs. low MD adherence). Further analysis suggested that this relationship was driven by vegetable and reduced/low/non-fat dairy consumption. Multivariable proportional odds models for being overweight or obese at baseline, and multivariable linear regression models comparing mean BMI z-score, percent body fat, waist circumference, waist-to-hip ratio, waist-to-height ratio, height at baseline, and height at menarche, did not show a significant relationship with MD adherence in this study. Multivariable linear mixed-effects growth models also did not show a difference in pubertal growth rate based on MD adherence.

The results of our study were consistent with previous findings that certain components of a Mediterranean-like diet were associated with a later age at menarche and thelarche. To our knowledge, this is the first study to demonstrate an association between an overall MD pattern and puberty outcomes. Our results suggest that consuming a more Mediterranean-like diet (high in plant-based foods, unsaturated fats, reduced/low/non-fat dairy, and fish, and low in red and processed meats), may decrease girls' risk of earlier puberty. Later age at puberty onset, in turn, could lead to decreased risk of adverse psychological, behavioral, and clinical outcomes in these girls, including low self-esteem, drug abuse, polycystic ovarian syndrome, and cancer risk. Further research is necessary to confirm our findings in other U.S. pediatric populations, and to elucidate the mechanism through which Mediterranean-like diet may influence puberty.

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Overall Introduction:

Background:

Puberty marks the change to a reproductive state, and for females, begins with breast development (thelarche) and/or the appearance of pubic hair (pubarche), and progresses to other physical changes such as a growth spurt and the onset of menstruation (menarche). In the past century, there has been a population-level trend toward earlier puberty in females in the United States and some Western European countries.[1] This trend may differ for the components of puberty; there is evidence that average age at thelarche has decreased more in recent years than average age at menarche.[1,2] Earlier development of secondary sex characteristics may impact a child's self-esteem and psychological and emotional well-being. Children may feel different from their peers, there may be a distorted perception of mental and behavioral maturity, and there may be unwanted attention from both peers and potentially abusive adults.[3] In turn, this could lead to drug and alcohol abuse,[4-6] teenage pregnancy,[7] and poor academic performance.[8] Early puberty has also been associated with clinical health risks, including increased risk for metabolic syndrome and polycystic ovarian syndrome.[3] Both earlier thelarche[9] and earlier menarche[10] have been associated with an increased risk of breast cancer, the most common cancer and second highest cause of cancer deaths among women.[11] For these reasons, better understanding factors related to earlier puberty could have a significant impact on public health.

Endocrinology and determinants of puberty:

The exact mechanism which triggers the onset of puberty remains unknown, but the hypothalamic-pituitary-gonadal (HPG) axis, which is inactive for most of childhood, becomes active during the time period directly preceding puberty. Pulsatile secretion of gonadotropin-releasing hormones (GnRHs) by neurons in the hypothalamus[12] stimulates gonadotropic cells in the pituitary to synthesize and release luteinizing hormone (LH) and follicle-stimulating hormone (FSH), the presence of which increases the production of sex hormones and triggers breast development (thelarche). About one year prior to thelarche, girls typically experience more frequent and higher nocturnal peaks of LH. At thelarche, the amplitude of LH peaks increases about tenfold, while the amplitude of FSH about doubles, and average estradiol increases.[12] At that point, girls are said to have reached Tanner stage 2 of breast development, based on a commonly used scale of physical development first developed by Marshall and Tanner.[13] There is positive feedback of sexual steroids on FSH, and estradiol levels continue to rise through progression to Tanner stage 4 of breast development, which is usually around the time of menarche.[14] After menarche, FSH is inhibited by sex steroids, and although cyclic uterine bleeding occurs, ovulation usually does not occur until an estradiol induced LH surge is established, about a year post-menarche.[12,14] During the time when production of sex steroids increases, the adrenal glands also begin secreting androgens, leading to axillary and pubic hair development, which occurs for 80% of girls around breast Tanner stage 3, but via a separate process.[12]

A specific trigger for the peripubertal increase in secretion of GnRHs is unknown. Several research groups have established a role for kisspeptin in the release of GnRH, and the administration of kisspeptin in prepubertal and adult mammals induces GnRH and gonadotropin release. Primate studies have also found kisspeptin to be released in a pulsatile fashion, with increasing amplitude and frequency upon puberty, along with GnRH release.[12] Glutamate, growth factors including IGF-1, and inhibition of gamma-aminobutyric acid (GABA) have also been found to be associated with GnRH release and expression.[12] Leptin, which is produced by the adipose tissue and signals nutritional status to the hypothalamus, has been extensively studied and is generally regarded as a permissive factor in the onset of puberty, although it does not have a direct effect on GnRH.[12] As such, leptin may also be an intermediary in the observed relationship between fat mass and the onset of puberty, discussed below.[15] Insulin may also play a role, most likely through kisspeptin neuron function.[16]

In addition to these proximate causes which have been linked to puberty initiation, other ultimate factors in the timing of puberty have been explored. Studies have found a high correlation between mother's and daughter's age at menarche,[17,18] and it is suggested that heredity accounts for 50-80% of the variance in puberty timing.[19] Thus, it is important to account for this hereditary component, if possible, when examining the role of other factors. Previous studies have shown socioeconomic factors during childhood, including family income and parental education, to be independent predictors of age at thelarche and menarche.[20-22] Racial differences in age at menarche, which cannot be fully explained by differences in other factors such as weight or socioeconomic differences, have also been observed.[23] Other early life exposures, including consumption of soy based formula[24,25] and low birth weight,[26] and social factors, including peer influence, psychosocial stress,[27] and an absent father,[28] have been associated with the timing of puberty as well. Environmental exposures, ranging from passive smoking[25] to endocrine disrupting chemicals (EDCs), may also influence puberty onset, and previous research has suggested a relationship between polychlorinated aromatic hydrocarbons (PCAHs) and delayed breast development,[29] phthalate esters and premature thelarche,[30] and phenols, phthalates, and phytoestrogens with breast and pubic hair staging and menarche.[31-33] Estrogenic mycotoxins, present in plant foods via fungal contamination and animal products via direct administration of zeranol, have also been associated with timing of breast development.[34]

The role of a critical body mass on puberty initiation, first proposed by Frisch,[35] is well recognized and centers on the idea that fat mass is a signal that promotes the release of estrogens and the initiation of maturation. This hypothesis has been supported by studies that found age at menarche to be inversely related to energy intake,[36] BMI,[37] and childhood obesity,[37-40] and both thelarche[37] and pubarche[41] to also be inversely related to BMI. Conversely, studies have found age at menarche to be positively related to physical activity (energy expenditure).[42] However, the relationship between factors such as BMI, energy intake, physical activity, and markers of puberty is complex.

Diet and Puberty:

Diet has also been proposed to play a role in puberty timing, but previous research in this area has focused mainly on the associations between specific foods or nutrients and puberty. Higher intakes of monounsaturated fatty acids,[43] fiber,[44,45] vegetable calories,[46] and nuts[47] have been associated with later menarche in some studies, while other studies have shown no association. High intakes of red meat[48] and omega-3 fatty acids[49] have been associated with earlier menarche in U.S. girls, but these results also require further confirmation. With regard to dietary patterns, early studies showed a vegetarian diet[50] and a pattern consistent with a vegetarian diet (meat analogues, nuts, grains, and beans)[51] to be associated with later age at menarche. Since greater energy intake has been inversely associated with age at menarche as discussed above, and food/nutrient intakes and dietary patterns are generally correlated with total energy intake, adjusting for energy intake is important in epidemiologic studies of diet in order to control for potential confounding and reduce extraneous variation.[52]

Mediterranean-like Diet:

The relationship between a Mediterranean-like diet and various health outcomes in adults and children has been researched in recent years. A "Mediterranean diet" generally refers to the type of diet traditionally consumed by people in Mediterranean countries such as Spain, Greece, and Italy. More recently, increased affluence in Mediterranean countries and globalization of the food supply has diversified the available food choices, which has had seemingly opposite consequences in Mediterranean and non-Mediterranean countries.[53] Traditionally "non-Mediterranean" foods, high in saturated fats and sugars and low in micronutrients, have become more available[54,55] and more popular[56] in Mediterranean countries, while fruit and vegetable intake has decreased;[57] consequently, the populations of these countries no longer consume a traditional “Mediterranean diet.” Conversely, foods such as olive oil, vegetables, fruits, nuts, and fish are becoming more available[54] and more popular in non-Mediterranean countries. While the precise definition of a Mediterranean or Mediterranean-like diet varies across studies, it generally denotes a diet high in plant-based foods (such as whole grains, vegetables, fruits, nuts, and legumes), unsaturated fats, and fish, and low in red and processed meats and high-fat dairy products. Research suggests a Mediterranean-like diet offers a reduction in all-cause mortality[58] and obesity[59,60] as well as a reduction in incidence and mortality of cardiovascular, cerebrovascular, and neoplastic diseases including cancer.[58,61]

Current evidence on a Mediterranean-like diet and pubertal outcomes, body fatness, and growth:

Dietary factors such as adherence to a Mediterranean-like diet may play a role in not only the onset of puberty, but also growth, body fatness, and obesity. We performed a thorough review to examine the current literature on a Mediterranean-like diet in relation to puberty, body fatness, and growth. While girls from Mediterranean countries have similar menarcheal ages to those from East and South Asian countries, they have a later age at thelarche.[1] To our knowledge, no study to date has evaluated the relationship between a Mediterranean-like diet and puberty timing. Higher intakes of certain Mediterranean-like dietary components including monounsaturated fatty acids,[43] fiber,[44,45] vegetable calories,[46] and nuts,[47] have been associated with later menarche in some studies, while high intakes of both red meat[48] and omega-3 fatty acids[49] (found in fish, a component of a Mediterranean-like diet) have been associated with earlier menarche in studies of U.S. girls. Overall, the current evidence suggests that a Mediterranean-like dietary pattern (high in plant-based foods and unsaturated fats, and low in red meat and high-fat dairy) may be associated with a later onset of puberty, which was the hypothesis we aimed to evaluate in this study.

Diet could also indirectly affect puberty by affecting BMI, and childhood obesity, on its own, can have harmful effects including increased risk of diabetes and heart disease, respiratory and joint problems, social and psychological problems, and increased risk of adult obesity (which is also a risk factor for many cancers).[62-64] Diet plays an essential role in the prevention of childhood obesity, and intervention on the level of overall dietary patterns may be particularly achievable by parents.

The current literature on the association between a Mediterranean-like diet and obesity or BMI in pediatric populations is inconsistent. Roughly half of around 35 studies in boys and girls have demonstrated an inverse relationship between a Mediterranean-like diet and BMI/obesity, while half have shown no association; a few more studies have demonstrated a positive relationship or one modified by other factors such as physical activity and parental education. However, most previous research studies have been cross-sectional in nature, and only two previous studies have been performed in pediatric populations in the United States.[65,66]

Growth, another indicator of pediatric health status, also changes markedly around the time of puberty. Taller height during childhood and adolescence, as well as increase in height during puberty, has been linked to health outcomes later in life, including breast cancer risk.[67-69] Although diet is one factor that plays a strong role in growth, previous research has not examined a Mediterranean-like diet in relation to peripubertal growth. A few previous cross-sectional studies did contain data on height across Mediterranean-like diet adherence groups, with some seeing no relationship, and some finding greater height with higher adherence to a Mediterranean-like diet. Further details of the literature review are presented in Chapters 1, 2, and 3 below.

In summary, previous research exploring the role of a Mediterranean-like diet in growth, body fatness, and puberty has been inconsistent. No previous studies have investigated a Mediterranean-like diet with regard to puberty timing and outcomes, nor have any studies examined height or peripubertal growth as a primary outcome. While some studies have shown an inverse association between consumption of a Mediterranean-like diet and increased BMI/obesity in pediatric populations, others have shown no association. Previous research on this topic in the U.S. is limited to only one study in a healthy pediatric population, which examined the outcome of BMI change over time.

Objectives of this dissertation project:

The results of this study contribute to filling a gap in knowledge on the topic of a Mediterranean-like diet and growth, body fatness, and puberty in girls, particularly in the United States. Improving awareness of dietary patterns associated with earlier puberty, as well as obesity, can lead to approaches to modify girls' diet and decrease their risks of obesity and earlier puberty (and in turn their risk of the aforementioned psychological, behavioral, and clinical sequelae). Given the aforementioned gaps in research, the purpose of this dissertation project was to evaluate the role of adherence to a Mediterranean-like diet in peripubertal growth, body fatness, and puberty in a cohort study of New Jersey girls, the *Jersey Girl Study.* We developed an index for assessing adherence to a Mediterranean-like diet in this population, and evaluated its relationship with both cross-sectional and longitudinal outcomes measured in the study, while adjusting for potential confounders. Levels of adherence to a Mediterranean-like diet were evaluated in relation to development status at baseline, timing of breast development, and timing of first menstruation. Additionally, the relationships between Mediterranean-like diet adherence and body mass and composition, as well as obesity status, were also investigated. Finally, height and growth rate during puberty were compared across Mediterranean-like diet adherence groups. Our results shed further light on the potential to modify these health outcomes by changing dietary patterns.

Overall Methods:

Study population:

The Jersey Girl Study has been described in detail elsewhere.[34] In brief, the *Jersey Girl Study* is a longitudinal cohort study based at the Rutgers Cancer Institute of New Jersey (PI: Dr. Elisa Bandera). The sample consists of 202 girls, age 9 or 10 at baseline (follow-up for growth and puberty outcomes ended in June 2018). Eligibility included residing in New Jersey, living with their biological mothers, no cognitive impairments, the ability to speak and read English, no major medical or surgical conditions known to affect their ability to thrive, stature, amenorrhea, or the timing of puberty; twins were also excluded although siblings were permitted. Participants were recruited primarily through pediatric practices, complemented with other methods including flyers in public places and word of mouth, between 2006 and 2014. This work utilized cross-sectional baseline data, as well as longitudinal follow-up data on growth and puberty outcomes. The study has approval from the Rutgers University Health Sciences Institutional Review Board.

Overall data collection:

General questionnaire:

The baseline general questionnaire from the *Jersey Girl Study* covered a range of factors including socioeconomic status, race/ethnicity, social factors and family structure, medical history, medication use, mother's age at menarche, age of onset of the girl's menarche and thelarche (if applicable), date of last menstrual cycle (if applicable), use of vitamins and organic foods, environmental factors, prenatal and early childhood factors, and physical activity. The questionnaire was filled out by the girls' biological mothers (or other guardian in few cases), with the assistance of the girls and study staff, if necessary.

Dietary intake assessment:

Diet history information was collected using three 24-hour recalls conducted by the Dietary Data Entry Center (DDEC) at Cincinnati Children's Hospital Medical Center. The data were collected on the intake of two weekdays and one weekend day, through direct interaction between the DDEC staff and the girls and their mothers via telephone. The DDEC uses the Nutrition Data System developed and maintained by the Nutrition Coordinating Center at the University of Minnesota. Personally identifying information from the girls was kept strictly confidential. Upon completion of the three interviews, dietary assessment and nutrient analyses were completed and provided to the staff of the *Jersey Girl Study.* This method of collecting dietary information has been established as providing a good estimate of energy intake in children.[70] The multiple recalls help to control for intra-individual dietary variability, and the open, semi-quantitative format also assists in providing an accurate representation of the girls' dietary intake. The information on the dietary report included descriptions and quantities of specific foods consumed, as well as the nutritional information and energy intake for each eating occasion.

Body measurement and puberty assessment:

Trained members of the *Jersey Girl Study* staff recorded the girls' height, weight, and waist and hip circumferences at the baseline study visit. These measurements were used to compute body mass index and waist-to-hip ratio. BMI for age and gender percentiles was calculated using the Centers for Disease Control and Prevention online calculator (BMI Percentile Calculator for Child and Teen).[71] Percent body fat was also measured to the nearest 0.1% using a bioelectrical impedance scale (Tanita corp., Tokyo, Japan).

Puberty was evaluated using the Tanner scale, which ranges from stage 1 (prepubertal) to stage 5 (post-pubertal).[13] Tanner stage was first reported by the girls' mothers using a form with pictorial representations and descriptions of each stage. They reported the age at thelarche (in months and years) for girls who had begun breast development. A physician also examined the girls and filled out the same Tanner staging form using palpation.

Annual Follow-Up:

Follow-up information was collected annually via mail by a form filled out by girls, their mothers, or both. The follow-up form asked if girls had reached menarche, and if so, what date; if girls' breasts had started to develop, and if so, at what age (years and months); current height and weight; and Tanner staging (the same forms as were filled out by each girl's mother at baseline).

Assessment of Mediterranean-like diet for this study:

Mediterranean Diet assessment in pediatric populations:

Previous studies have evaluated the intake of a Mediterranean-type diet in pediatric populations.[72] The majority of studies used the KIDMED index,[73] which calculates a score based on the answers to 16 yes/no questions about eating habits and consumption of different food groups. It includes not only information on traditional components of the Mediterranean diet, such as fruit, nuts, vegetables, grains, fish, and olive oil, but also asks about sweet and candy consumption, fast food intake, and breakfast consumption and composition. Some studies used the KIDMED questionnaire directly, while others imputed the information using other information such as that from a food frequency questionnaire.

A thorough literature search also yielded 20 papers that used some version of a Mediterranean Diet Score, another method of assessment that has been used in adult populations,[74] to assess Mediterranean diet adherence in children. This type of index categorizes food groups as beneficial or detrimental components, and uses a median value of consumption in the study sample for each group as a cutoff to assign a point for levels either above the cutoff for beneficial components, or below the cutoff for detrimental components. The total of points is then summed to yield a total score, and categories of this total MDS index can be used for analysis purposes. As variations of this method of assessment have been widely used in the pediatric literature, and it is well-suited to the dietary recall data available from the *Jersey Girl Study*, we derived a Mediterranean-like diet score to quantify intake of a Mediterranean-like diet in our study sample.

Development of a Mediterranean-like diet index for this study:

Based on the indices used in previous research studies (discussed above), we developed a Mediterranean Diet Score-type index of ‘beneficial’ and ‘detrimental’ diet components. Food groups included as ‘beneficial’ components were vegetables (not potatoes), legumes, nuts, fruits, whole grains, fish, reduced fat/low-fat/fat free dairy, and unsaturated: saturated fat ratio (see details in Table I). Included as a 'detrimental' component was red or processed meat intake. One point was given for an intake above the median intake of the study sample for each beneficial category, or below the median intake for each detrimental category, and the points were summed to give a total score which ranged from 0 to 9. Although intake of legumes and nuts was low (median intakes of 0g/day and 0.000333 g/day, respectively), it was decided not to combine the categories since it seemed that it was not the same girls who were consuming legumes and nuts. Fish consumption in the sample was also very low (median intake of 0g/day). Thus, for the calculation of the index, 1 point was given for eating *any* legumes, *any* nuts, and *any* fish. The details of the median consumption of each food group in the sample, and criteria for index calculation, are included Table I.

Foods Included in Each Category:

Details of foods included and excluded in each beneficial and detrimental category are included in Table I below. Briefly, most vegetables and 100% vegetable juices, excluding potatoes, were included in a vegetable category. Fruits and 100% fruit juices were included in a fruit category. Legumes were included in a separate category, excluding soy. Nuts and seeds including nut and seed butters were included in their own category. Whole grain or partial whole grain foods were included in the fiber-rich whole grain category if they have a fiber to total carbohydrate ratio of 0.11 (1.1 g of fiber to 10 g of carbohydrates)[75]; cakes, cookies, pies, pastries, doughnuts, and snack bars were excluded regardless of fiber to total carbohydrate ratio. Fish included fresh, smoked, and fried fish. Dairy included reduced fat/low fat/nonfat milk, flavored milk, flavored milk beverage powders, cheese, and yogurt, and excluded full fat dairy, cream, dairy desserts, dairy based meal replacements or supplements, infant formula, nondairy milk, nondairy cheese, and nondairy desserts. (Some previous studies included dairy as a detrimental component, but these typically referred to full-fat dairy products). Finally, the detrimental category of red or processed meat included beef, pork, veal, lamb, cured pork, game, cold cuts, sausage, organ meats, and meat based savory snacks, but not poultry.

Statistical analyses:

We calculated summary statistics (counts and percent for categorical variables, and mean or median and standard deviation for continuous variables) for the characteristics of the study participants (demographics, socioeconomic factors, Mediterranean-like diet index scores, anthropometric measurements, puberty outcomes, and potential confounders) for all girls, and for those who had high vs. medium vs. low adherence to a Mediterranean-like diet (p-values for differences were computed using chi-square tests, ANOVA, and Brown-Mood tests, as well as Fisher's exact test when necessary). The Mediterranean-like diet score was used both as a continuous variable, as well as divided into low (0-3), medium (4-5), and high (6-9) adherence groups, with the cutoffs set based roughly on tertiles of the score in the total study sample. Since Mediterranean-like diet score = 4 comprised almost 23% of the sample on its own, sensitivity analysis was run with adherence groups re-categorized as 0-3, 4, and 5-9. Results were nearly the same, but differences between groups were slightly less clear.

Statistical analyses specific to each analysis are detailed in the respective chapters. All analyses were conducted using SAS version 9.4.

Characteristics of Study Sample:

The distributions of demographic and socioeconomic characteristics of the entire study sample are presented in Table II. Overall, girls had their baseline study interview at a mean of 10.02 years of age. They were physically active, exceeding the CDC-recommended one hour of physical activity per day, with a mean of 8.84 hours of physical activity per week; physical activity level did not differ significantly across Mediterranean-like diet adherence groups. The study sample was predominantly (88%) White and affluent, with 69% of families having an income of > $100,000 per year, and only 9% of families having an income under $70,000 per year. Mothers were highly educated, with 34% having a Bachelor's as their highest degree, and 46% having a Master's degree or higher level of education. These demographic and socioeconomic factors did not differ by diet adherence group. Total energy intake was significantly different across Mediterranean-like diet adherence groups, with the lowest median daily energy intake (1557.68 kcal) in the low adherence group, and the highest median daily energy intake in the high adherence group (1727.95 kcal, $p=0.03$). Finally, dietary energy density (excluding water) also differed across diet adherence groups, with lower energy density (kcal energy/g food) with higher adherence to a Mediterranean-like diet.

Results specific to each analysis are detailed and discussed in the following chapters.

Table I - Food Group Categories for the Mediterranean-like Diet Index

Food group	Included	Excluded	Median Value (g/day)	Range (g/day)	Percentage Consumers (any)	Criteria for Index Calculation
Beneficial Components:						
Vegetables	• Dark green vegetables (raw, cooked, or canned) • Deep yellow vegetables (raw, cooked, or canned) • Tomatoes (includes salsa, tomato sauce, puree, paste, and raw, cooked, or canned) • Other vegetables (raw, cooked, or canned) • Other starchy vegetables (raw, cooked, or canned) • 100% vegetable juice	• Ketchup • Potatoes (any) • Vegetable-based savory snacks • Not 100% vegetable juice • Olives • Pickles & pickled vegetables • Clamato juice	90.58	0.00-356.47	98.51%	1 point if consumption 90.58 g/day or higher
Legumes	• Cooked dried beans • Refried beans • Peas • Lima beans	• Soy based desserts • TVP • Soy nuts • Tofu • Tempeh	0	0.00-186.73	37.62%	1 point if ate any legumes
Nuts	• Nuts • Seeds • Nut butters • Seed butters		0.000333	0.00-72.56	50.00%	1 point if ate any nuts
Fruits	• 100% fruit juices • Citrus fruit (fresh,	• Not 100% fruit juice • Jams/jellies • Fruit in	177	0.00-854.92	97.03%	1 point if consumption 177 g/day or higher

Food group	Included	Excluded	Median Value (g/day)	Range (g/day)	Percentage Consumers (any)	Criteria for Index Calculation
	canned, or cooked) • Other fruits (fresh, frozen, cooked, canned, or dried) • Avocados	baked goods, candy, granola bars, sweets, or ice cream • Maraschino cherries • Fruit leather				
Fiber-rich whole grains*	• Grains, flours & dry mixes • Bread • Rolls • Quick breads & tortillas • Crackers • Pastas • Ready to eat cereal • Popcorn	• Cakes • Cookies • Pies • Pastries • Doughnuts • Snack bars	16.5	0.00-200.20	74.75%	1 point if consumption 16.5 g/day or higher
Fish	• Fresh fish • Smoked fish • Canned fish • Fried fish		0	0.00-74.69	26.73%	1 point if ate any fish
Dairy	• Milk (reduced fat, low fat, nonfat) • Ready to drink flavored milk (reduced fat, low fat, nonfat) • Sweetened flavored milk beverage powders with non-fat dry milk • Cheese (reduced fat, low fat, nonfat) • Yogurt	• Full fat milk, flavored milk, cheese, or yogurt • Dairy desserts • Frozen dairy desserts • Cream • Dairy-based meal repl. or supplement • Infant formula • Nondairy milk • Nondairy cheese • Frozen	214.4	0.00-1138.99	97.52%	1 point if consumption 214.4 g/day or higher

Food group	Included	Excluded	Median Value (g/day)	Range (g/day)	Percentage Consumers (any)	Criteria for Index Calculation
	(reduced fat, low fat, nonfat)	nondairy dessert				
Unsaturated fats (MUFA + PUFA) to Saturated fats Ratio	• Total monounsatu-rated fatty acids + total polyunsatur-ated fatty acids to total saturated fatty acids		1.53	0.74-3.33	N/A	1 point if ratio is 1.53 or higher
Detrimental Components:						
Red and processed meats	• Beef • Veal • Lamb • Pork • Cured pork • Game • Cold cuts/ sausage • Organ meats • Meat based savory snacks	• Poultry	40.41	0.00-157.96	85.64%	1 point if consumption was **less** than 40.41 g/day
* as described above, whole grain or partial whole grain foods were included if they had a fiber to total carbohydrate ratio of 0.11 (1.1 g of fiber to 10 g of carbohydrates); excluded foods were excluded regardless of whole grain to carbohydrate ratio						

Table II - Subject Characteristics, Overall and According to Mediterranean-like Diet Adherence Score

	Total (n=202)	**Low MD Adherence (MDS 0-3) (n=72, 35.64%)**	**Med. MD Adherence (MDS 4-5) (n=85, 42.08%)**	**High MD Adherence (MDS 6-9) (n= 45, 22.28%)**	**P-value**
Child age at baseline (mean (SD))	10.02 (0.58)	10.09 (0.63)	9.97 (0.54)	9.97 (0.54)	0.36
Child age at baseline (median)	10.07	10.18	10.00	9.96	0.48
Race, n (%)					0.09
White	177 (87.62)	65 (90.28)	76 (89.41)	36 (80.00)	
Other	22 (10.89)	6 (8.33)	7 (8.24)	9 (20.00)	
Income (per year), n (%)					0.48
< $70,000	19 (9.41)	8 (11.11)	9 (10.59)	2 (4.44)	
$70,000-$84,999	13 (6.44)	3 (4.17)	8 (9.41)	2 (4.44)	
$85,000-$99,999	15 (7.43)	5 (6.94)	8 (9.41)	2 (4.44)	
$100,000+	140 (69.31)	53 (73.61)	53 (62.35)	34 (75.56)	
Mother's education, n (%)					0.46
High school-Associate degree	41 (20.30)	13 (18.06)	21 (24.71)	7 (15.56)	
Bachelor's degree	69 (34.16)	29 (40.28)	24 (28.24)	16 (35.56)	
Master's degree	61 (30.20)	23 (31.94)	24 (28.24)	14 (31.11)	
Professional/doctoral degree	31 (15.35)	7 (9.72)	16 (18.82)	8 (17.78)	
Other caretaker's education,* n (%)					0.86
High school-Associate degree	18 (8.91)	4 (5.56)	9 (10.59)	5 (11.11)	
Bachelor's degree	44 (21.78)	16 (22.22)	17 (20.00)	11 (24.44)	
Master's degree	32 (15.84)	12 (16.67)	11 (12.94)	9 (20.00)	
Professional/doctoral degree	23 (11.39)	8 (11.11)	7 (8.24)	8 (17.78)	
Total physical activity (mean hours per week (SD))	8.84 (6.28)	8.80 (5.41)	9.35 (6.78)	7.93 (6.64)	0.48
Total physical activity (median hours per week)	7.06	7.26	7.20	5.89	0.40
Total energy intake, kcal (mean (SD))	1718.77 (384.77)	1625.02 (369.62)	1773.74 (385.96)	1764.94 (382.80)	**0.03**
Total energy intake, kcal (median)	1677.98	1557.68	1710.40	1727.95	**0.03**
Dietary energy density (kcal energy/g food) (mean (SD))	0.915 (0.181)	0.916 (0.177)	0.916 (0.175)	0.912 (0.200)	0.99
Dietary energy density (kcal energy/g food) (median)	0.911	0.908	0.909	0.915	0.98
Dietary energy density (kcal energy/g food) (excluding g water) (mean (SD))	4.569 (0.183)	4.607 (0.198)	4.582 (0.165)	4.482 (0.163)	**0.0009**
Dietary energy density (kcal energy/g food) (excluding g water) (median)	4.561	4.578	4.592	4.466	**0.03**

* Not all mothers indicated another caretaker. Categories may not total to 100% due to missing values.

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Chapter 1: Mediterranean-like Diet and its Relationship with Puberty Outcomes

Introduction:

Research has shown evidence of a trend of earlier puberty in American and Western European girls, which may be even more pronounced for breast development compared to first menstruation.[1,2] Whether this earlier thelarche represents true hypothalamic–pituitary–gonadal axis activation, is gonadotropin-independent puberty, or is the result of obesity, exposure to exogenous estrogens or endocrine disrupting chemicals, earlier development of secondary sexual characteristics may impact a child's self-esteem and psychological well-being. These effects on mental and emotional health may in turn be linked to drug and alcohol abuse,[3-5] teen pregnancy,[6,7] and poor academic performance.[8,9] Earlier puberty also presents clinical health risks, including increased risk for metabolic syndrome and polycystic ovarian syndrome,[10] and earlier thelarche[7] and menarche[9] have both been associated with breast cancer risk.

Diet is just one factor that influences puberty timing. Intake of specific foods and nutrients, including animal proteins, has been linked to earlier puberty, while intake of other foods like sugar-sweetened beverages suggests an association but requires more research.[11] Studying food patterns rather than isolated foods or nutrients gives a better summary of subjects' eating habits, and also reflects potential interactions among different nutrients. A Mediterranean-like diet, generally defined as one high in plant-based foods (such as whole grains, vegetables, fruits, nuts, and legumes), unsaturated fats, and fish, and low in red and processed meat and high-fat dairy, has been studied widely in relation to various health outcomes. The current evidence on a Mediterranean-like diet in relation to puberty is described below.

Previous research on components of a Mediterranean-like diet in relation to puberty:

Although the relationship between a Mediterranean-like diet and puberty has not specifically been evaluated to date, previous research has touched on the relationship between some components of a Mediterranean-like eating pattern and puberty outcomes. Higher intakes of monounsaturated fatty acids (MUFAs), a typical distinguishing feature of a Mediterranean-like diet, by peripubertal girls have been associated with a later age at menarche ($p < 0.05$, after adjustment for age and energy intake, among Canadian girls with a mean age of 10.7 years).[12] However, in a longitudinal cohort study of British girls, there was no evidence of an association between MUFA intake and occurrence of menarche.[13] In addition to monounsaturated fats, a Mediterranean-like diet tends to contain high levels of omega-3 fatty acids due to intake of foods such as fish. A higher intake of omega-3 fatty acids at age 10 has been associated with earlier menarche in U.S. girls (RR of 2.7 (95% confidence interval (CI) 1.6-4.6), $p=0.03$).[14]

A Mediterranean-like diet is also typically high in fiber due to high consumption of fruits, vegetables, legumes, and whole grains (in our study we specifically used only fiber-rich whole grains as part of our diet index, as discussed in the overall methods above). Several studies have explored the association between prepubertal fiber consumption and puberty timing, and found menarche to be delayed in girls with high childhood fiber intakes (relative hazard of 0.54, 95% CI 0.31-0.94 after energy adjustment, in a study of premenarcheal Canadian girls).[15,16] However, other studies found no association between peripubertal fiber intakes and menarche or breast development.[12,17] Consumption of nuts and seeds is another characteristic of a Mediterranean-like diet, and in a cross-sectional study of Spanish girls was inversely related to menarche in girls older than age twelve (OR = 0.71, CI=0.40-0.98).[18] Finally, Mediterranean-like diets typically include low intakes of red meats. There is also evidence that a high childhood intake of red meat (age 5-12 years) is associated with earlier age at menarche (HR 1.64, 95% CI 1.11-2.41, p-trend <0.001).[19] Since there is some evidence that components of a Mediterranean-like diet (such as intake of MUFAs, fiber, and nuts) are related to later or lesser odds of menarche, and that intake of red meat is associated with earlier age at menarche, it follows that the Mediterranean-like dietary pattern as a whole may be associated with a delayed onset of puberty.

It is also possible that a Mediterranean-like diet is associated with puberty via its influence on BMI. Some previous research has shown an inverse association between consumption of a Mediterranean-like diet and obesity in pediatric populations, and childhood obesity has also been associated with earlier menarche.[20] Attaining a critical body mass and/or fat mass has been speculated as a permissive factor in the onset of puberty, with leptin being a possible link between body fat and stimulation of gonadotropins at both the hypothalamic and pituitary level, and studies have observed that higher BMI was associated with an early menarche[21] as well as thelarche.[21] The relationship between a Mediterranean-like diet and childhood body composition and obesity in this sample will be explored further in Chapter 2.

Summary:

There have been no studies to date that have investigated the direct association between a Mediterranean-like diet pattern as a whole and puberty in children or adolescents. This project aimed to address these gaps in the research. We hypothesized that greater adherence to a Mediterranean-like diet would be associated with reduced odds of thelarche at baseline, a later age at thelarche, and greater time to menarche in our analyses.

Methods:

Details about the study population and overall data collection procedures have been outlined in the introductory section. A brief overview and additional information pertinent to this chapter's specific research questions are included below.

In brief, the *Jersey Girl Study* is a longitudinal cohort study based at the Rutgers Cancer Institute of New Jersey (PI: Dr. Elisa Bandera).[22] The sample consists of 202 girls, age 9 or 10 at baseline. Eligibility included residing in New Jersey, living with their biological mothers, no cognitive impairments, the ability to speak and read English, no major medical or surgical conditions known to affect their ability to thrive, stature, amenorrhea, or the timing of puberty;

twins were also excluded although siblings were permitted. Data were collected from baseline and follow-up questionnaires filled out by girls' mothers, as well as an initial baseline study appointment, and three 24-hour dietary recalls.

Data collection:

Dietary intake assessment:

Dietary data were collected using three 24-hour recalls conducted by the Dietary Data Entry Center (DDEC) at Cincinnati Children's Hospital Medical Center, as described in detail in the overall methods section. The data were collected on different days of the week, through direct interaction between the DDEC staff and the girls' mothers via telephone.

Assessment of Mediterranean-like diet for this study:

In brief, variations of a Mediterranean Diet Score have been used widely in the pediatric literature. This type of index categorizes food groups as beneficial or detrimental components, and uses the median value of consumption in the study sample for each component as a cutoff to assign a point for levels either above the cutoff for beneficial components, or below the cutoff for detrimental components. The total of points is then summed to yield a total score, and categories of this total MDS index can be used for analysis purposes.

Based on the indices used in previous research studies we developed a Mediterranean Diet Score-type index of 'beneficial' and 'detrimental' diet components. Food groups included as 'beneficial' components were vegetables (not potatoes), legumes, nuts, fruits, whole grains, fish, reduced fat/low-fat/fat free dairy, and unsaturated to saturated fat ratio (see details in Table I). Included as a 'detrimental' component was red meat intake. One point was given for an intake above the median intake of the study sample for each beneficial category, or below the median intake for each detrimental category, and the points were summed to give a total score which ranges from 0 to 9. The specific types of foods included in each category are detailed in Table I of the introductory section.

Assessment of Puberty Outcomes:

Thelarche at Baseline:

Puberty was evaluated using the Tanner scale, which ranges from stage 1 (prepubertal) to stage 5 (post-pubertal).[23] Tanner stage was reported by the girls' mothers using a form with pictorial representations and descriptions of each stage; a physician also examined the girls and filled out the same form at the baseline study interview. Thelarche at baseline was defined as a Tanner stage of 2 or greater, as reported by the physician (or as reported by the mother in the 16 girls who did not have physician staging). A previous analysis of agreement between mother and physician Tanner staging for breast development found agreement to be high (over 85% agreement, kappa 0.7).[24]

Age at Thelarche:

Age at thelarche was determined using two different questions. First, at the baseline eligibility interview, as well as on each follow-up form, mothers were asked if girls' breasts had started to develop, and if so, at what age. Second, mothers were asked to provide an update on puberty staging using the Tanner scale, as discussed above, at baseline and each successive annual follow-up (until menarche). Age at thelarche was based purely on mothers' and not physician report, since physician validation was only available at the time of baseline interview. If the mother reported breast development at baseline, and reported an age of breast development, this was used as age at thelarche. When age at thelarche was missing or there were inconsistencies in the mother's report (n=54), we determined age at thelarche according to the following rules. If the mother reported a Tanner stage of 2 or greater before the reported age at thelarche (or age at thelarche was missing), the age at report of Tanner stage 2 (or 6 months before, in cases of girls 10 or older at report), was used as an estimate of the correct age at thelarche. If a mother estimated an age in years but not months, the age in years plus 6 months was used. If the mother's estimate at the subsequent follow-up was similar but more precise, that estimate of age was used.

Age at Menarche:

Most girls did not reach menarche until after the baseline visit, due to study eligibility criteria. Date of first period was asked during the baseline eligibility interview, and on each subsequent annual follow-up questionnaire. If only the month and year was provided, the 15th of the month was imputed as the date of first menstrual period.

Statistical analyses:

We calculated summary statistics (counts and percent for categorical variables, and mean or median and standard deviation for continuous variables) for the distribution of puberty outcomes and covariates for all girls, and for those who had high vs. medium vs. low adherence to a Mediterranean-like diet (p-values for differences were computed using chi-square tests, ANOVA, and Brown-Mood tests, as well as Fisher's exact tests when necessary). Binomial regression models were fit to compute prevalence ratios (PRs) and 95% confidence intervals (CI) to examine the association between adherence to a Mediterranean-like diet and thelarche at baseline. Since the binomial regression models did not converge, we used Poisson regression models with an independent correlation structure to approximate the model.[25] Logistic regression models were also fit to compute odds ratios (ORs) and 95% CIs, for comparison purposes. In addition to evaluating a Mediterranean-like dietary pattern, the influence of specific food groups on thelarche was explored by fitting both separate models and models which were mutually adjusted for other food groups that were part of the Mediterranean-like diet score for our study. Linear regression models were fit to compare predicted mean age at thelarche based on girls' adherence to a Mediterranean-like diet. While age at thelarche was assessed as described above, using mothers' and/or daughters' reports, a sensitivity analysis was performed excluding those girls whose Tanner staging at baseline was not in agreement with physician-assessed Tanner staging. Cox proportional hazards models were fit to compute hazard ratios (HRs) and 95% CIs to examine the association between adherence to a Mediterranean-like diet and time to menarche (using age as the time scale). The influence of specific food groups on menarche was also further explored by fitting separate models for each of the food groups that were part of our Mediterranean-like diet score.

Potential confounders of interest that were considered for adjustment in the multivariable models included: age at baseline (continuous), race (white or other), annual household income (<$70,000, $70,000- $84,999, $85,000-$99,999, > $100,000), mother's education (high school-associate degree, bachelor's degree, master's degree, professional/doctoral degree), total energy intake (kcal/day), and total hours of girl's physical activity per week. Additionally, as there is a hereditary component to puberty timing, maternal age at thelarche/menarche was considered for inclusion in the respective models. Further, girl's BMI (using BMI z-score) was considered for inclusion in models and examined as a potential modifier of any relationship seen between diet and puberty outcomes. Model covariates were selected based on a priori hypotheses, as well as statistical significance levels in the models.

Results:

Puberty outcomes in the sample, as well as the distribution of covariates in the models, are reported in Table 1.1. Girls' BMI and BMI category, and mother's age at menarche, did not differ according to Mediterranean-like diet adherence group. Nearly 60% of girls (n=120) had begun breast development (Tanner stage 2 or higher) at their baseline visit. Although not statistically significant, girls with high adherence to a Mediterranean-like diet were less likely to have reached thelarche at baseline and tended to have later age at thelarche and menarche.

The results of the Poisson regression models for thelarche at baseline in relation to Mediterranean-like diet adherence are presented in Table 1.2. In the full model (adjusted for age at baseline, baseline total energy intake, BMI z-score at baseline, and mother's age at thelarche), high adherence to a Mediterranean-like diet (MDS score 6-9) was associated with 35% lower prevalence of thelarche at baseline (prevalence ratio (PR) 0.65, 95% confidence interval (CI) 0.48-0.90, p=0.0087). Adherence to a Mediterranean-like diet on a continuous scale also suggested a lower prevalence of thelarche at baseline per unit increase in adherence score, though this was not statistically significant (PR 0.95, 95% CI 0.89-1.01). Results from a similar logistic regression model (presented in Table 1.3) showed a similar and even stronger association than the Poisson models (OR 0.22 (95% CI 0.08-0.59), p=0.003 for high vs. low adherence, and OR 0.79 (95% CI 0.64-0.98), p=0.03 for continuous adherence score).

Since a significant association was seen between overall Mediterranean-like diet adherence and thelarche, additional models were fit to further investigate the relationship of the specific dietary components of our Mediterranean-like diet score and thelarche (data are presented in Supplemental Tables 1a and 1b). Fish consumption was significantly inversely associated with thelarche (PR = 0.77) in a fully adjusted model which was mutually adjusted for the other Mediterranean-like diet components (p=0.02). High vs. low consumption of reduced/low/non-fat dairy showed a significant inverse relationship with thelarche (PR = 0.75) in a fully adjusted model which was mutually adjusted for the other components (p=0.008). Logistic regression models for the specific diet components showed similar significant inverse associations with thelarche for fish consumption and reduced/low/non-fat dairy consumption (data shown in Supplemental Table 1b).

The results of linear regression models for mean age at thelarche are presented in Table 1.4. Though not statistically significant, the fully adjusted model (adjusted for age at baseline, baseline total energy intake, BMI z-score at baseline, and mother's age at thelarche) showed a trend toward a later age at thelarche with higher Mediterranean-like diet adherence (mean age of 10.42 vs. 10.05 for high vs. low adherence). Our sensitivity analysis, which excluded those girls whose Tanner staging at baseline was not in agreement with physician-assessed Tanner staging, showed similar results; there was no statistically significant effect of diet adherence on age at thelarche (data not shown). The results of Cox proportional hazards models (using age as the time scale) for time to menarche are presented in Table 1.5. Results from the fully adjusted model (adjusted for age at baseline, race, baseline total energy intake, physical activity, BMI z-score at baseline, and mother's age at menarche) showed a significantly longer time to menarche for girls in the high vs. low diet adherence group (hazard ratio (HR) 0.45, 95% CI 0.28-0.71, p=0.0009). Similarly, adherence to a Mediterranean-like diet on a continuous scale showed significantly longer time to menarche per unit increase in diet adherence score (HR 0.89, 95% CI 0.82-0.98, p=0.01). Further exploring the individual diet components showed vegetable consumption and reduced/low/non-fat dairy consumption to be significantly related to time to menarche in a fully adjusted model which was mutually adjusted for the other components (HRs 0.66 (95% CI 0.46-0.95), p=0.02, and 0.63 (95% CI 0.43-0.91), p=0.01, respectively). These data are presented in Supplemental Table 1c.

Discussion:

In this analysis, high adherence to a Mediterranean-like diet, when compared to low adherence, was associated with significantly reduced odds of thelarche at baseline, in accordance with our hypothesis. Supplemental analyses suggest that this association may be particularly driven by these girls' higher consumption of fish and reduced/low/non-fat dairy. Although the results were not statistically significant, our models also suggested that there was a corresponding trend toward later age at thelarche with higher Mediterranean-like diet adherence. High adherence to a Mediterranean-like diet when compared to low adherence, as well as continuous Mediterranean-like diet adherence score, were also associated with greater time to menarche, which was also in agreement with our initial hypotheses. Our results also suggest that this relationship may be driven by higher consumption of vegetables and reduced/low/non-fat dairy.

Our study is the first to investigate the relationship between adherence to a Mediterranean-like diet and puberty outcomes. Our results are consistent with previous findings that associated higher intakes of monounsaturated fatty acids (MUFAs),[12,15] fiber,[15,16] and low-fat dairy,[26] and lower intake of red meat,[19] all components of our diet adherence index, with a later age at menarche. However, although higher adherence to a Mediterranean-like diet was associated with greater time to menarche in our sample, intake of unsaturated fats, fiber, and red meat did not show independent associations. A British study[13] did not find evidence of an association between MUFA intake and menarche, and other studies found no association between peripubertal fiber intake and menarche.[12,17] This suggests that the previously observed relationship between MUFA and fiber intake and age at menarche may have actually represented the effect of an overall dietary pattern associated with high unsaturated fat and fiber intake, such as a Mediterranean-like diet. A previous study of Spanish girls showed evidence of an inverse relationship between nut and seed consumption and menarche,[18] which was not shown in our sample and may likewise have been the effect of an overall dietary pattern. Consumption of nuts and seeds was low in our population, which may have affected our power to detect an association. Although a previous study found a high intake of omega-3 fatty acids to be associated with earlier menarche in girls,[14] this did not seem to impact time to menarche in our sample, perhaps because fish consumption overall was low (with a median intake in the sample of 0 grams per day).

Our analysis indicated that consumption of reduced/low/non-fat dairy was independently associated with later age at both thelarche and menarche, which was in accordance with another recent prospective study of Chilean girls that found low-fat dairy to be associated with later age at menarche.[26] However, several previous studies did not find a significant association between dairy or low-fat dairy consumption in children and age at menarche.[13,19,27,28] A prospective study of pre-pubertal girls in Iran found a higher risk of earlier menarche in girls with higher milk intake, although the relationship was not significant for cheese or yogurt consumption.[29] In the U.S., Wiley found weak evidence that greater milk intake was associated with earlier age at menarche based on National Health and Nutrition Examination Survey (NHANES) data.[30] Thus, the association between different types of dairy intake and age at thelarche and menarche requires further study, particularly since milk composition and hormone levels may vary across different regions and countries.[31,32]

Since a critical body or fat mass has been widely regarded as a permissive factor in puberty onset, all of our models were adjusted for girl's BMI (z-score) to account for potential mediating effects. We did not see a relationship between BMI z-score and Mediterranean-like diet adherence in this study sample (see further details on this in Chapter 2 below). However, BMI z-score was independently associated with a 40% increase in the prevalence of thelarche at baseline (per unit change in z-score), as well as positively associated with mean age at thelarche ($p<0.0001$, data not shown). No interaction effect was seen between girl's BMI and Mediterranean-like diet adherence in our models.

Puberty initiation is accompanied by a release of gonadotropins, decreased levels of sex-hormone binding globulin (SHBG), and increased levels of estrogens, and early pubertal plasma estrogen levels predict the rate of pubertal development. Consuming a Mediterranean-like diet has been associated with higher levels of SHBG[33] and lower levels of endogenous estrogens.[34] Previous research also indicates that increased intake of dietary fiber (a component of a Mediterranean-like diet) may reduce circulating bioavailable estrogen levels[16,26] as well as follicle stimulating hormone and luteinizing hormone levels (gonadotropin production).[16] The proposed mechanisms for the influence of a Mediterranean-like diet on SHBG and hormone levels mainly center around the effects of fiber and phytoestrogens, which concurs with our findings that the relationship between diet and menarche may be driven by consumption of vegetables. Previous research suggests that fiber inhibits the reabsorption of steroid hormones in the gut, leading to increased excretion of estrogens from the body.[35] Phytoestrogens have also been implicated in stimulating liver synthesis of SHBG, which reduces biological availability of sex hormones.[36] Thus, the observed impacts of a Mediterranean-like diet and its components on SHBG and hormone levels provide a plausible mechanism for the influence of such a diet on the timing of puberty, as we found in this study.

Strengths of our study include prospectively collected data on age at menarche, which reduced the possibility of recall bias. Data on age at thelarche were collected retrospectively for some girls, but still within about 2 years at most, which made significant recall errors unlikely. Thelarche was assessed at the baseline study appointment by a physician, and was likely to be accurate. Dietary data were representative of the peripubertal period and occurred prior to menarche. The dietary recall interviews allowed for capturing intake of exact foods consumed, and represented both weekday and weekend consumption. The study questionnaire and anthropometric measurements taken also provided us with a wide range of potentially relevant confounding variables for adjustment in our regression models.

Several limitations should also be acknowledged. First, the sample was predominantly White, with generally highly educated mothers, which may limit the generalizability of the findings to other populations. As with many other studies of diet and puberty, some of our analyses were cross-sectional and could have resulted in reverse causation, as over half of girls had already begun puberty at baseline when the dietary recall was done. However, the fact that the cross-sectional results are in the same direction as our longitudinal analyses provides reassurance. Some girls were lost to follow-up before age at menarche could be ascertained (n=31, 15.3%), which may have potentially introduced follow-up bias. The sample size was relatively small (n=200), which limited the statistical power of some analyses. Furthermore, diet was only assessed at baseline, and therefore we assumed that dietary patterns remained unchanged for our analysis period.

The results of this study contribute to filling a gap in knowledge on the topic of a Mediterranean-like diet and puberty in girls in the United States. Higher adherence to a Mediterranean-like diet was associated with later thelarche as well as menarche in our study. Modifying dietary pattern is more easily accomplished than implementing specific foods or nutrients. Our results support our hypothesis that following a Mediterranean-like dietary pattern may decrease girls' risk of earlier puberty. In turn, this would impact their risk of psychological, behavioral, and clinical consequences, including drug abuse, teen pregnancy, metabolic syndrome, and breast cancer risk. However, future research is necessary to confirm these findings in other populations.

Table 1.1: Puberty Outcomes and Selected Covariates, Overall and According to Mediterranean-like Diet Adherence

	Total (n=202)	**Low MD Adherence (MDS 0-3) (n=72, 35.64%)**	**Med. MD Adherence (MDS 4-5) (n=85, 42.08%)**	**High MD Adherence (MDS 6-9) (n= 45, 22.28%)**	**P-value**
Child age at baseline, years (mean (SD))	10.02 (0.58)	10.09 (0.63)	9.97 (0.54)	9.97 (0.54)	0.36
Breast Development at baseline, n (%)					0.14
Yes (Tanner stage 2+)	120 (59.41)	46 (63.89)	53 (62.35)	21 (46.67)	
No (Tanner stage 1)	82 (40.59)	26 (36.11)	32 (37.65)	24 (53.33)	
Age at Thelarche (mean (SD))	10.19 (1.27)	10.25 (1.34)	10.07 (1.18)	10.30 (1.30)	0.52
Age at Thelarche (median)	10.00	10.00	9.94	10.04	0.75
Age at Menarche (mean (SD))	12.76 (1.39)	12.80 (1.37)	12.56 (1.33)	13.08 (1.48)	0.18
Age at Menarche (median)	12.74	12.69	12.40	13.18	0.29
Race, n (%)					0.09
White	177 (87.62)	65 (90.28)	76 (89.41)	36 (80.00)	
Other	22 (10.89)	6 (8.33)	7 (8.24)	9 (20.00)	
Total physical activity (mean hours per week (SD))	8.84 (6.28)	8.80 (5.41)	9.35 (6.78)	7.93 (6.64)	0.48
Total energy intake, kcal (mean (SD))	1718.77 (384.77)	1625.02 (369.62)	1773.74 (385.96)	1764.94 (382.80)	**0.03**
Child BMI, kg/m^2 (mean (SD))	18.04 (3.10)	17.80 (3.27)	18.28 (3.01)	17.98 (3.01)	0.63
Mother's age at thelarche, n (%)					**0.01**
Before age 8	1 (0.50)	0 (0.00)	1 (1.18)	0 (0.00)	
Between ages 8 and 12	120 (59.41)	37 (51.39)	52 (61.18)	31 (68.89)	
After age 12	51 (25.25)	26 (36.11)	21 (24.71)	4 (8.89)	
Missing	30 (14.85)	9(12.5)	11(12.94)	10(22.22)	
Mother's age at menarche, n (%)					0.85
Before age 12	39 (19.31)	12 (16.67)	19 (22.35)	8 (17.78)	
Age 12-14	128 (63.37)	46 (63.89)	54 (63.53)	28 (62.22)	
Age 14 or later	26 (12.87)	10 (13.89)	9 (10.59)	7 (15.56)	

Categories may not total to 100% due to missing values; p-values for means from ANOVA; for medians from Brown-Mood; for categories from chi-square; for mom's age at thelarche from Fisher's exact test

Table 1.2: Poisson Regression Analysis for Thelarche at Baseline (Yes vs. No) Related to Mediterranean-like Diet Adherence

	Prevalence Ratio (95% confidence interval)	
	Model adjusted only for age	**Fully adjusted model***
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	Ref.	Ref.
Medium adherence (4-5)	1.03 (0.82-1.29)	0.92 (0.74-1.14)
High adherence (6-9)	0.77 (0.54-1.10)	**0.65** **(0.48-0.90)**§
Adherence to a Mediterranean-like diet (continuous score)	0.99 (0.92-1.05)	0.95 (0.89-1.01)

*Full model is adjusted for age at baseline, girl's total energy intake, girl's BMI z-score at baseline, and mother's age at breast development

§ $p<0.01$

Table 1.3: Logistic Regression Analysis for Thelarche at Baseline (Yes vs. No) Related to Mediterranean-like Diet Adherence

	Odds Ratio (95% confidence interval)	
	Model adjusted only for age	**Fully adjusted model**
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	Ref.	Ref.
Medium adherence (4-5)	1.04 (0.53-2.05)	0.56 (0.24-1.28)
High adherence (6-9)	0.52 (0.24-1.15)	**0.22 (0.08-0.59)** §
Adherence to a Mediterranean-like diet (continuous score)	0.95 (0.80-1.13)	**0.79 (0.64-0.98)**ǂ

*Full model is adjusted for age at baseline, girl's total energy intake, girl's BMI z-score at baseline, and mother's age at breast development

Interaction term between BMI and diet adherence was not significant.

§ $p<0.01$, ǂ $p<0.05$

Table 1.4: Mean Age at Thelarche and 95% Confidence Intervals According to Mediterranean-like diet Adherence, Estimated from Multiple Linear Regression as Described Below

	Mean Age at Thelarche (95% confidence interval)	
	Model adjusted only for age	**Fully adjusted model***
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	10.25 (9.95-10.55)	10.05 (9.77-10.33)
Medium adherence (4-5)	10.07 (9.80-10.34)	10.15 (9.88-10.41)
High adherence (6-9)	10.30 (9.92-10.68)	10.42 (10.07-10.77)
Adherence to a Mediterranean-like diet (per unit increase)	-0.02 (-0.13, 0.08)	0.06 (-0.04-0.15)

*Full model is adjusted for age at baseline, girl's total energy intake, girl's BMI z-score at baseline, and mother's age at breast development

Table 1.5: Hazard Ratios and 95% Confidence Intervals for Time to Menarche (on an age time-scale) from a Cox Proportional Hazards Model, According to Mediterranean-like Diet Adherence

	Hazard Ratio (95% confidence interval)	
	Model adjusted only for age at baseline	**Fully adjusted model***
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	Ref.	Ref.
Medium adherence (4-5)	1.14 (0.81-1.61)	0.98 (0.67-1.45)
High adherence (6-9)	0.72 (0.48-1.09)	**0.45 (0.28-0.71)**§
Adherence to a Mediterranean-like diet (continuous)	0.98 (0.90-1.06)	**0.89 (0.82-0.98)**ǂ

*Full model is adjusted for age at baseline, race, total energy intake, physical activity, girl's BMI z-score at baseline, and maternal age at menarche

Interaction term between BMI and diet adherence was not significant.

§ $p<0.01$, ǂ $p<0.05$

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Chapter 2: Mediterranean-like Diet and its Relationship with Peripubertal Body Fatness

Introduction:

As mentioned in Chapter 1, diet may potentially have an indirect effect on puberty outcomes through body composition and BMI. Studies have observed an inverse relationship between BMI and age at menarche as well as thelarche.[1] There are several possible explanatory relationships; high BMI may be an initiating factor for earlier puberty, while puberty may also predispose girls to weight gain (and potentially obesity). Childhood obesity itself is a matter of public health concern, which may lead to harmful effects including increased risk of diabetes and heart disease, respiratory and joint problems, social and psychological problems, and increased risk of adult obesity (which is also a risk factor for many cancers).[2-4] The peripubertal period is especially crucial, since there is evidence that both adherence to healthy dietary patterns and physical activity levels tend to decline as children reach adolescence. While other genetic and environmental factors may contribute to obesity, diet may play an important role. More so than the investigation of specific foods or nutrients, information on the role of overall dietary patterns may be easier for parents to interpret and translate into practice. Previous research on the association between a Mediterranean-like diet and BMI or obesity in pediatric populations has been inconsistent but generally tended to show an inverse association. Only two of these studies were conducted in the United States, and only one of them was in a healthy population of children (8-15 years old).[5] The current state of the research is outlined below.

Previous research on Mediterranean-like diet in relation to BMI and body fatness:

Over 35 previous research studies have evaluated the association between a Mediterranean-like diet and body composition or obesity in pediatric populations, but BMI or obesity were not always primary outcomes, and some of the measures were only correlations or unadjusted statistical tests. Most of these were short-term, cross-sectional or interventional studies from Mediterranean countries like Greece,[6-16] Italy,[17-24] Croatia,[25] Cyprus,[26] and Spain,[27-31] but there were also a few prospective cohorts,[5,14,30,32-34] and research from Britain,[35] Mexico,[36] Portugal,[34] Chile,[37] Finland,[38] Lithuania/Serbia,[39] and a European consortium[32] as well as two studies from the U.S.[5,33] The majority of these studies used the KIDMED questionnaire[40] to evaluate adherence to a Mediterranean-like diet. The KIDMED index[40] calculates a score based on the answers to 16 yes/no questions about eating habits and consumption of different food groups. It includes information on traditional components of the Mediterranean diet, such as fruit, nuts, vegetables, grains, fish, and olive oil, but also asks about sweet and candy consumption, fast food intake, and breakfast consumption and composition. A few studies of Mediterranean-like diet and BMI or body composition in children used some version of a Mediterranean Diet Score (MDS) [41] to assess Mediterranean-like diet adherence. This type of index categorizes food groups as beneficial or detrimental components, and uses a median value of consumption in the study sample for each group as a cutoff to assign a point for levels either above the cutoff for beneficial components, or below the cutoff for detrimental components. The total of points is then summed to yield a total score, and categories of this total index can also be used for analysis purposes.

Over a dozen studies, including several specifically in girls, found an inverse relationship between a Mediterranean-like diet and BMI or overweight/obesity status.[5,7,10,16,21,25,28,31,32,36,37,39,42,43] However, over a dozen studies, some in girls, did not demonstrate a significant relationship between Mediterranean-like diet adherence and BMI or obesity,[8,9,13,15,19,22,23,26,29,30,33-35,38] and two studies even indicated a positive association[14,20] between Mediterranean-like diet adherence and BMI or obesity. Furthermore, seven studies showed an inverse association between Mediterranean-like diet adherence and waist circumference,[6,9,27,32,37,42,44] while four studies did not demonstrate any association,[8,28,33,38] and one study found a positive relationship between KIDMED score and waist circumference.[14] Finally, five studies found an inverse relationship between adherence to a Mediterranean-like diet and fat mass or percent body fat,[25,32,36,37,42] while a few other studies did not find any significant association between Mediterranean diet and body composition.[8,15,29] A summary table on the details of these previous studies can be found in Appendix B.

Studies of BMI or obesity outcomes:

One of the two U.S. studies on this topic found an inverse association between a Mediterranean dietary pattern and BMI change in a pediatric population. Martin-Calvo, et al. used data from the Growing Up Today Study II of 10,918 girls and boys ages 8-15 from across the United States, who were recruited children of women in the Nurses' Health Study II.[5] The study found that a 2-point increase in KIDMED score was associated with a lower gain in BMI ($p=0.001$) in their repeated-measures GEE model adjusted for important factors such as sex, BMI, and physical activity.[5] In the subgroup analysis of girls only ($n=6,002$), they also found a 2-point increase in KIDMED score to be associated with a lower gain in BMI ($p=0.06$).[5] A cross-sectional analysis of Italian adolescent girls ages 11-16 also found high adherence to a Mediterranean diet, as measured by KIDMED score, to be significantly associated with lower odds of being overweight or obese (OR 0.66, 95% CI 0.47-0.93 from the adjusted logistic regression model) and inversely associated with BMI ($p=0.001$).[17] Two cross-sectional studies of girls in Spain[28,31] both found significant differences in Mediterranean diet adherence by overweight/obesity status (both $p < 0.05$). A cohort study of children ages 2-9 from 8 European countries also found high adherence to a Mediterranean-like diet (based on MDS score) to be inversely associated with overweight or obesity (OR 0.85, 95% CI 0.77-0.94 from adjusted logistic regression model); high MDS at baseline was also found to be inversely associated with increase in BMI.[32]

Cross-sectional studies of youth and adolescents have yielded significant odds ratios ranging from 0.24[16] to 0.74[39] for the inverse relationship between Mediterranean diet adherence and overweight and/or obesity, even after adjustment for factors such as gender, age, SES, physical activity, energy intake, and parents' education. Other cross-sectional studies also found significant inverse correlations or associations between Mediterranean diet adherence and BMI.[43,7,25,37,45] A 2014 interventional study of children with median age of 11 in Mexico found that the Mediterranean diet intervention group had significantly decreased BMI (p=0.001).[36] An analysis of 10-12 year old children from the Greek PANACEA study found an inverse relationship between Mediterranean-like diet adherence assessed by KIDMED and obesity status, but only among families with at least one parent of a high education level (> 15 years or tertiary/postgraduate education) in adjusted regression models (OR 0.41, 95% CI 0.17-0.98).[10] A previous analysis of this study had found no significant association between KIDMED score and BMI or overweight/obesity in adjusted regression models.[13]

The other U.S. study on this topic looked at 793 children under age 20 with type 1 diabetes in the SEARCH nutrition ancillary cohort study, comprised of multiple study centers across the U.S.[33] Children in their analytical sample were a mean age of 13-14 years old, approximately 45% female, and around 75% were non-Hispanic White.[33] This study did not find a relationship between Mediterranean diet adherence (assessed by modified KIDMED score) and BMI, in models adjusted for important potential confounders such as age, sex, race, education, income, and physical activity.[33] A Portuguese cohort study of teenagers also did not show a significant association between Mediterranean diet adherence and BMI in girls (p=0.57),[34] nor did a cross-sectional study of Finnish girls ages 6-8 (p=0.86).[38] A cohort study of adolescents in Spain also did not find a relationship between Mediterranean diet adherence and BMI category, after adjusting for age, gender, and socioeconomic status.[30] Numerous cross-sectional analyses of peripubertal children in Greece [8,13,15], Italy[19,22,23], Spain[29], and Britain[35] did not find a statistically significant relationship between Mediterranean diet adherence and BMI or obesity. There was also no such relationship found among older (ages 12-18[6], 15-17[7], 12-19[22]) children in several more cross-sectional studies. Lazarou, et al. used national data from peripubertal children in Cyprus and found an inverse association between KIDMED score and overweight/obesity, but this association disappeared after their logistic regression model was adjusted for physical activity (OR 0.20, 95% CI 0.021-1.86).[26]

Only two previous studies have demonstrated some type of positive relationship between adherence to a Mediterranean-type diet and BMI or obesity. In 2014, Santomauro, et al. studied adolescents ages 14-20 in Florence, Italy and found being normal weight, overweight, or obese to be inversely associated with poor KIDMED score (OR 0.47, 95% CI 0.24-0.94 for overweight/obese).[20] Also, a 2011 cohort study of Greek adolescents ages 12-17 found KIDMED score to be positively related to BMI (r=0.122, p=0.02), although this was not a primary endpoint of the study.[14]

Studies of body fat distribution:

A statistically significant inverse association was seen for Mediterranean diet adherence and waist circumference in female Italian adolescents,[17] Greek children,[6,7,9] Chilean children,[37] and Spanish adolescents,[27] even after adjustment for factors such as age and height. Among a cohort of children ages 2-9 from 8 European countries, high adherence to a Mediterranean-like diet at baseline was found to be inversely associated with increase in waist circumference and waist-to-height ratio (OR 0.87, 95% CI 0.77-0.98, and OR 0.88, 95% CI 0.78-0.99, respectively).[32] The U.S. study examining youth with type 1 diabetes in the SEARCH nutrition ancillary study did not find a relationship between Mediterranean diet adherence and waist circumference in adjusted models.[33] Other studies also observed no significant association between Mediterranean diet adherence and anthropometric measurements such as waist circumference among Greek schoolchildren,[6] Spanish schoolchildren,[26] and 6-8 year old Finnish girls.[38] One cohort study of Greek adolescents found a positive relationship between KIDMED score and waist circumference (r=0.118, p=0.02), although this was not a primary endpoint of the study.[14]

Studies of body composition:

Fat mass was inversely related to Mediterranean diet adherence in cross-sectional observational studies of Italian adolescent females[17] and Croatian university students,[25] and percent body fat was inversely associated with KIDMED score in a study of 10-11 year old Chilean children.[37] The cohort of European children ages 2-9 also found high adherence to a Mediterranean-like diet to be inversely associated with percent fat mass (beta=-0.22, 95% CI -0.43 , -0.01 from adjusted linear regression model).[32] An interventional study of children in Mexico found that the Mediterranean diet intervention group had significantly decreased fat mass (p=0.001).[36] However, several studies of 10-12 year old children in Mediterranean countries did not observe a significant association between Mediterranean diet adherence and percent body fat.[8,15,29]

Summary:

The relationship observed between a Mediterranean-like diet and obesity and body composition among children has been inconsistent. Furthermore, previous research in a U.S. pediatric population thus far has been limited to two studies, one of which was among a specific population of children with type 1 diabetes. The only study in a healthy pediatric population found an inverse association between a Mediterranean-like diet and BMI. Both measured and unmeasured confounders such as environmental and lifestyle factors may differ in American children as compared to those from other countries. Foods consumed which are consistent with a Mediterranean-type diet may also differ greatly in the U.S. compared to other areas. Thus, further research in this population is necessary, and this project aimed to address this gap in the research. We hypothesized that we would find an inverse relationship between adherence to a Mediterranean-like diet and BMI, overweight/obesity, percent body fat, and waist circumference in our sample of peripubertal girls.

Methods:

Details about the study population and overall data collection procedures have been outlined in the introductory section. A brief overview and additional information pertinent to this chapter's specific research questions are included below.

In brief, the *Jersey Girl Study* is a longitudinal cohort study based at the Rutgers Cancer Institute of New Jersey (PI: Dr. Elisa Bandera).[46] The sample consists of 202 girls, age 9 or 10 at baseline. Eligibility included residing in New Jersey, living with their biological mothers, no cognitive impairments, the ability to speak and read English, no major medical or surgical conditions known to affect their ability to thrive, stature, amenorrhea, or the timing of puberty; twins were also excluded although siblings were permitted. Data were collected from baseline and follow-up questionnaires filled out by girls' mothers, as well as an initial baseline study appointment, and three 24-hour dietary recalls.

Data collection:

Dietary intake assessment:

Dietary data were collected using three 24-hour recalls conducted by the Dietary Data Entry Center (DDEC) at Cincinnati Children's Hospital Medical Center, as described in detail in the overall methods section. The data were collected on different days of the week, through direct interaction between the DDEC staff and the girls' mothers via telephone.

Assessment of Mediterranean-like diet for this study:

In brief, variations of a Mediterranean Diet Score have been used widely in the pediatric literature. This type of index categorizes food groups as beneficial or detrimental components, and uses a median value of consumption in the study sample for each group as a cutoff to assign a point for levels either above the cutoff, for beneficial components, or below the cutoff, for detrimental components. The total of points is then summed to yield a total score, and categories of this total MDS index can be used for analysis purposes.

Based on the indices used in previous research studies, we developed a Mediterranean Diet Score-type index of ‘beneficial’ and ‘detrimental’ diet components. Food groups included as ‘beneficial’ components were vegetables (not potatoes), legumes, nuts, fruits, whole grains, fish, reduced fat/low-fat/fat free dairy, and unsaturated to saturated fat ratio (see details in Table I). Included as a ‘detrimental’ component was red meat intake. One point was given for an intake above the median intake of the study sample for each beneficial category, or below the median intake for each detrimental category, and the points were summed to give a total score which ranges from 0 to 9. The specific types of foods included in each category are detailed in Table I of the introductory section.

Anthropometric Measurements:

Trained members of the *Jersey Girl Study* staff recorded girls’ height, weight, and waist and hip circumferences at the baseline study visit. These measurements were used to compute body mass index (weight in kilograms divided by the square of height in meters), waist-to-hip ratio, and waist-to-height ratio. Percent body fat was also measured to the nearest 0.1% using a bioelectrical impedance scale (Tanita Corp., Tokyo, Japan).

Statistical analyses:

We calculated summary statistics (counts and percent for categorical variables, and mean or median and standard deviation for continuous variables) for the distribution of BMI and anthropometric measurement outcomes and covariates for all girls, and for those who had high vs. medium vs. low adherence to a Mediterranean-like diet (p-values for differences were computed using chi-square tests, ANOVA, and Brown-Mood tests). Girls were categorized as underweight, normal weight, or overweight/obese based on age- and sex-specific BMI percentile cutoffs published by the CDC.[47] Proportional odds models were fit to compute odds ratios (ORs) and 95% confidence intervals (CI) to examine the association between adherence to a Mediterranean-like diet and odds of being overweight/obese at baseline. Logistic regression models were also fit to compute odds ratios (ORs) and 95% CI for the odds of being overweight or obese at baseline (vs. underweight or healthy weight), for comparison purposes. Separate linear regression models were fit to compare predicted mean BMI z-score, waist circumference, waist-to-hip ratio, waist-to-height ratio, and percent body fat based on girls' adherence to a Mediterranean-like diet. BMI-for-age percentiles and z-scores were calculated using the Centers for Disease Control and Prevention (CDC) SAS program based on CDC growth charts.[48]

Potential confounders of interest that were considered for adjustment in the multivariable models included: age at baseline (continuous), race (white or other), annual household income (<\$70,000, \$70,000-\$84,999, \$85,000-\$99,999, > \$100,000), mother's education (high school-associate degree, bachelor's degree, master's degree, professional/doctoral degree), total energy intake (kcal/day), and total hours of girl's physical activity per week. Additionally, as there is a hereditary component to obesity, maternal overweight/obesity based on self-reported height and weight was considered for inclusion in the models. Finally, parental education (both maternal education and that of another caregiver, if listed), was evaluated as a potential effect modifier, as some previous studies have shown a different relationship between Mediterranean-like diet and BMI, depending on parents' education. Model covariates were selected based on a priori hypotheses, as well as statistical significance levels in the models.

Results:

Anthropometric measurements and body mass index (BMI) in the study sample, as well as the distribution of covariates in the models, are reported in Table 2.1. Girls in the study had a mean baseline BMI of 18.04 (standard deviation (s.d.) 3.10), with 73% of girls being categorized as a healthy weight, and 21% of girls being categorized as overweight or obese. Girls' BMI and BMI category did not differ across Mediterranean-like diet adherence groups. On average, girls in the study had approximately 20% body fat, a waist circumference of 65.88 cm, a waist-to-hip ratio of 0.85, and a waist-to-height ratio of 0.47. Similarly to BMI, none of these anthropometric measurements differed significantly across diet adherence groups.

The results of a proportional odds model fit for odds of being overweight or obese at study baseline (vs. healthy and underweight) are presented in table 2.2. Neither the age adjusted model nor the full model adjusted for baseline age, physical activity, and maternal education showed a statistically significant relationship between Mediterranean-like diet adherence and BMI status (Odds Ratio (OR) 0.85 (95% CI 0.36-2.01) for high vs. low adherence in the fully adjusted model). Likewise, the OR confidence interval for continuous Mediterranean-like diet adherence score in these models included the null value (OR 1.05 (95% CI 0.87-1.26)). Fitting logistic regression models for odds of overweight/obese at baseline vs. underweight/healthy weight showed similar nonsignificant results (data shown in Table 2.3). Sensitivity analysis examining BMI z-score or BMI status as a predictor and Mediterranean-like diet adherence as the outcome showed no significant result (data not shown).

The results of linear regression models for mean BMI z-score are presented in table 2.4. Results from the crude model, as well as the full model adjusted for physical activity level and maternal education, did not show significant differences in BMI z-score at baseline based on diet adherence (predicted mean z-score 0.12 (-0.13-0.37) for the low adherence group vs. 0.22 (-0.10-0.53) for the high adherence group; predicted change per unit change in MDS score of 0.06 (-0.02-0.14)). Similarly, results of linear regression models for percent body fat (presented in table 2.5) did not show significant differences across diet adherence groups (predicted mean percent body fat 20.12 (18.00-22.22) for the low adherence group vs. 20.07 (17.44-22.71) for the high adherence group, from the full model adjusted for baseline age, physical activity level and maternal education; predicted change per unit change in MDS score of 0.32 (-0.39-1.03)). Finally, the results of linear regression models for mean waist circumference, waist-to-height ratio, and waist-to-hip ratio are presented in table 2.6. Age-adjusted and fully adjusted models (covariates for each outcome are listed in the table footnote) did not show a significant difference for any of these anthropometric measurements across diet adherence groups. The interaction term between maternal and/or other caretaker education and diet adherence was not significant in any of our models.

Discussion:

The results of this study did not show any evidence of an association between Mediterranean-like diet adherence on either a categorical or continuous scale, and BMI or overweight/obesity, in our sample of peripubertal girls. Mediterranean-like diet adherence also did not show any statistically significant association with measures of body composition and body fat distribution, such as percent body fat, waist circumference, waist-to-height ratio, or waist-to-hip ratio. Parental education did not appear to be an effect modifier in this study, as it was in some previous studies.

To date, about half of studies investigating the association between a Mediterranean-like diet and BMI or obesity in pediatric populations have shown an inverse relationship, while about half, like our study, showed no association. The literature on a Mediterranean-like diet in relation to measures such as waist circumference, fat mass, and percent body fat, has also been inconclusive. Among studies which have shown an inverse association, as well as those that have shown no association, are high quality, large studies that have used validated exposures and models adjusted for important potential confounders such as age, gender, physical activity, and energy intake. Previous research in U.S. pediatric population has been limited to two studies so far – a longitudinal study of children ages 8-15, which showed an inverse relationship between KIDMED score and gain in BMI in adjusted models,[5] and a study of children with type 1 diabetes, which did not find a relationship between KIDMED score and BMI in adjusted models.[33] The longitudinal study evaluated BMI change, under the rationale that self-reported weight may be underreported, but change calculated from self-reported weight is accurate; our study did not have to address this issue, as we had measured weight for all study participants from the same time period as their dietary measurement. This study also revised the original KIDMED index to better suit the American population (for instance, excluding sugary cereals and high fat dairy as beneficial components, which our study did as well), and adjusted their models for important potential confounders such as age, sex, baseline BMI, physical activity, sedentary time, and total energy intake.[5] Although the other pediatric study in the U.S. did not find a relationship between diet adherence and BMI, this was in a population of children and adolescents with type 1 diabetes, and the effects of diet may be different as compared to an average pediatric population.[33]

Though many of the previous studies, as well as ours, adjusted for important potential confounders, such as age, physical activity, socioeconomic status, and total energy intake, unmeasured confounders such as environmental and lifestyle factors may differ across populations, especially internationally. The foods and nutrients represented by what is consistent with a Mediterranean-like diet may also differ greatly between different cultures. The majority of studies constructed their exposure based on the KIDMED index, sometimes taken from a food frequency questionnaire, which could have presented information and recall bias issues. Furthermore, the KIDMED index includes not only information on traditional components of a Mediterranean-like diet, but also asks about sweet and candy consumption, fast food intake, and breakfast consumption and composition. Thus, using KIDMED score as the measure of diet adherence may result in different associations than using an alternative measure of diet adherence.

Several limitations of our study should be acknowledged, which may have hindered our ability to detect any associations. First, the sample was predominantly White, with highly educated mothers, which may limit the generalizability of the findings to other populations. The sample size was relatively small (n=200), which may have limited the statistical power to detect small differences in outcomes. There was also a low percentage of girls with a very high BMI (21% were overweight/obese, as compared to 32% of children age 10-17 in New Jersey),[49] which may have limited our ability to detect associations with BMI and obesity.

However, our study did have several strengths, including that height, weight, percent body fat, and other anthropometric measurements used as outcomes in this analysis were measured using a standardized protocol by trained research staff for all girls in the study. The dietary recall interviews allowed for capturing intake of exact foods consumed, and represented both weekday and weekend consumption. The study questionnaire and anthropometric measurements taken also provided us with a wide range of potentially relevant confounding variables for adjustment in our regression models.

The results of this study, which did not show any associations between adherence to a Mediterranean-like diet and body fatness in peripubertal girls, contribute to the continually growing body of research on the topic. Only one U.S. study so far has demonstrated an association between Mediterranean-like diet and BMI, and further research is needed to validate these findings, particularly among healthy children in the United States.

Table 2.1: BMI & Anthropometric Measurement Outcomes and Selected Covariates, According to Mediterranean-like Diet Adherence

	Total (n=202)	Low MD Adherence (MDS 0-3) (n=72, 35.64%)	Med. MD Adherence (MDS 4-5) (n=85, 42.08%)	High MD Adherence (MDS 6-9) (n= 45, 22.28%)	P-value
Child BMI, kg/m^2 (mean (SD))	18.04 (3.10)	17.80 (3.27)	18.28 (3.01)	17.98 (3.01)	0.63
Child BMI, kg/m^2 (median)	17.32	16.98	17.65	17.48	0.67
Child BMI category, n (%)					0.36
Underweight	12 (5.94)	7 (9.72)	2 (2.35)	3 (6.67)	
Healthy weight	147 (72.77)	49 (68.06)	64 (75.29)	34 (75.56)	
Overweight/obese	43 (21.29)	16 (22.22)	19 (22.35)	8 (17.78)	
Percent body fat (mean (SD))	20.03 (8.76)	19.58 (8.89)	20.54 (8.62)	19.79 (8.92)	0.78
Percent body fat (median)	19.10	18.60	19.15	19.10	0.98
Waist circumference (cm) (mean (SD))	65.88 (8.86)	65.79 (9.18)	66.43 (8.81)	65.00 (8.55)	0.68
Waist circumference (cm) (median)	63.85	62.98	64.45	63.27	0.44
Waist-to-Height Ratio (mean (SD))	0.47 (0.05)	0.47 (0.06)	0.48 (0.05)	0.46 (0.05)	0.48
Waist-to-Height Ratio (median)	0.46	0.46	0.47	0.46	0.58
Waist-to-Hip Ratio (mean (SD))	0.85 (0.05)	0.85 (0.05)	0.86 (0.05)	0.84 (0.04)	0.19
Waist-to-Hip Ratio (median)	0.85	0.85	0.86	0.84	0.30
Child age at baseline, years (mean (SD))	10.02 (0.58)	10.09 (0.63)	9.97 (0.54)	9.97 (0.54)	0.36
Mother's education, n (%)					0.46
High school-Associate degree	41 (20.30)	13 (18.06)	21 (24.71)	7 (15.56)	
Bachelor's degree	69 (34.16)	29 (40.28)	24 (28.24)	16 (35.56)	
Master's degree	61 (30.20)	23 (31.94)	24 (28.24)	14 (31.11)	
Professional/doctoral degree	31 (15.35)	7 (9.72)	16 (18.82)	8 (17.78)	
Total physical activity (mean hours per week (SD))	8.84 (6.28)	8.80 (5.41)	9.35 (6.78)	7.93 (6.64)	0.48
Total energy intake, kcal (mean (SD))	1718.77 (384.77)	1625.02 (369.62)	1773.74 (385.96)	1764.94 (382.80)	**0.03**

Categories may not total to 100% due to missing values; p-values for means from ANOVA; for medians from Brown-Mood; for categories from chi-square

Table 2.2: Proportional Odds Analysis for Odds of Being Overweight/Obese at Baseline (vs. Healthy/Underweight), Related to Mediterranean-like Diet Adherence

	Odds Ratio (95% confidence interval)	
	Model adjusted only for age	**Fully adjusted model**
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	Ref.	Ref.
Medium adherence (4-5)	1.30 (0.64-2.62)	1.20 (0.59-2.48)
High adherence (6-9)	0.88 (0.38-2.07)	0.85 (0.36-2.01)
Adherence to a Mediterranean-like diet (continuous)	1.04 (0.87-1.25)	1.05 (0.87-1.26)
*Full model is adjusted for age at baseline, physical activity, and maternal education		

Table 2.3: Logistic Regression Analysis for Odds of Being Overweight/Obese at Baseline (vs. Healthy/Underweight), Related to Mediterranean-like Diet Adherence

	Odds Ratio (95% confidence interval)	
	Model adjusted only for age	**Fully adjusted model**
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	Ref.	Ref.
Medium adherence (4-5)	0.96 (0.45-2.06)	1.01 (0.44-2.31)
High adherence (6-9)	0.72 (0.28-1.87)	0.74 (0.27-2.06)
Adherence to a Mediterranean-like diet (continuous)	1.02 (0.84-1.24)	1.05 (0.85-1.29)
*Full model is adjusted for age at baseline, physical activity, total energy intake, and maternal education		

Table 2.4: Mean BMI Z-score and 95% Confidence Intervals According to Mediterranean-like Diet Adherence, Estimated from Multiple Linear Regression as Described Below

	Mean BMI Z-score (95% confidence interval)	
	Crude model	**Fully adjusted model**
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	0.05 (-0.19-0.29)	0.12 (-0.13-0.37)
Medium adherence (4-5)	0.33 (0.11-0.55)	0.36 (0.14-0.59)
High adherence (6-9)	0.19 (-0.11-0.49)	0.22 (-0.10-0.53)
Change per unit change in adherence to a Mediterranean-like diet (continuous score)	0.07 (-0.02-0.15)	0.06 (-0.02-0.14)
*Full model is adjusted for physical activity and maternal education		

Table 2.5: Mean Percent Body Fat and 95% Confidence Intervals According to Mediterranean-like Diet Adherence, Estimated from Multiple Linear Regression as Described Below

	Mean Percent Body Fat (95% confidence interval)	
	Model adjusted only for age	**Fully adjusted model**
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	19.51 (17.45-21.56)	20.12 (18.00-22.22)
Medium adherence (4-5)	20.58 (18.66-22.50)	20.94 (19.04-22.85)
High adherence (6-9)	19.84 (17.22-22.46)	20.07 (17.44-22.71)
Change per unit change in adherence to a Mediterranean-like diet (continuous score)	0.37 (-0.34-1.08)	0.32 (-0.39-1.03)
*Full model is adjusted for age at baseline, maternal education, and physical activity		

Table 2.6: Mean Waist Circumference (cm), Waist-to-Height Ratio, and Waist-to-Hip Ratio, and 95% Confidence Intervals According to Mediterranean-like diet Adherence, Estimated from Multiple Linear Regression as Described Below

	Mean Waist Circumference (cm) (95% confidence interval)		**Mean Waist-to-Height Ratio (95% confidence interval)**		**Mean Waist-to-Hip Ratio (95% confidence interval)**	
	Model adjusted only for age	**Fully adjusted model***	**Model adjusted only for age**	**Fully adjusted model***	**Model adjusted only for age**	**Fully adjusted model***
Adherence to a Mediterranean-like diet						
Low adherence (0-3)	65.65 (63.59-67.71)	66.21 (64.07-68.35)	0.47 (0.46-0.48)	0.47 (0.46-0.48)	0.85 (0.84-0.86)	0.85 (0.84-0.86)
Medium adherence (4-5)	66.51 (64.61-68.40)	66.81 (64.91-68.71)	0.48 (0.46-0.49)	0.48 (0.47-0.49)	0.86 (0.85-0.87)	0.86 (0.85-0.87)
High adherence (6-9)	65.08 (62.47-67.68)	65.28 (62.64-67.92)	0.46 (0.45-0.48)	0.46 (0.45-0.48)	0.84 (0.82-0.85)	0.84 (0.82-0.85)
Change per unit change adherence to a Mediterranean-like diet score (continuous)	0.18 (-0.53-0.89)	0.13 (-0.59-0.85)	0.00010 (-0.0043-0.0045)	0.00049 (-0.004-0.005)	-0.0013 (-0.0055-0.0029)	-0.0017 (-0.0059-0.0026)

*Full model for waist circumference is adjusted for age at baseline, maternal education, and physical activity

*Full model for waist-to-height ratio is adjusted for age at baseline, total energy intake, and physical activity

*Full model for waist-to-hip ratio is adjusted for age at baseline and physical activity

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<u>Chapter 3: Mediterranean-like Diet and its Relationship with Peripubertal Growth</u>

Introduction:

Along with puberty staging, statural growth is another indication of a child's overall health status around the time of puberty, marked by an acceleration followed by a deceleration in growth. The pubertal growth spurt begins accordingly with the activation of the hypothalamic pituitary gonadal axis, and in girls, linear growth increases from about 5 cm/year to about 8.3 cm/year.[1] Peak growth rate generally occurs around one year after puberty begins (around Tanner stage 3), and 1.5 years before menarche. Height during adolescence may have implications for health outcomes later in life. For instance, shorter height at menarche has been associated with reduced premenopausal breast cancer risk in Caucasian women, after adjustment for important factors including age at menarche and family history of breast cancer.[2] Tallness throughout childhood[3] and high stature at 14 years of age, as well as increase in height during puberty,[4] have been associated with increased breast cancer risk. As with other events of growth and development, heredity is an important factor, but environmental influences such as diet also play a strong role.

Previous research on a Mediterranean-like diet in relation to peripubertal growth:

Previous studies have not examined the longitudinal effect of a Mediterranean-like diet on peripubertal growth. A handful of studies in children or adolescents provided some data comparing height measurements across adherence groups of a Mediterranean-like diet, though none of these studies were in the U.S., and height was not the primary study outcome. Some of these studies used the KIDMED index,[5] which calculates a score based on the answers to 16 yes/no questions about eating habits and consumption of different food groups, to evaluate adherence to a Mediterranean-like diet. The KIDMED index includes information on traditional components of the Mediterranean diet, such as fruit, nuts, vegetables, grains, fish, and olive oil, but also asks about sweet and candy consumption, fast food intake, and breakfast consumption and composition. The other studies used some version of a Mediterranean Diet Score (MDS)[6] to assess Mediterranean-like diet adherence. This score categorizes food groups as beneficial or detrimental components, and uses a median value of consumption in the study sample for each group as a cutoff to assign a point for levels either above the cutoff for beneficial components, or below the cutoff for detrimental components. The total of points is then summed to yield a total Mediterranean Diet Score.

Studies which did not see an association between Mediterranean-like diet and height:

In a cross sectional study of 6-8 year old children, Eloranta, et al. did not see any significant relationship between height and Mediterranean Diet Score among girls (p=0.248 from regression analysis) or boys (p=0.751).[7] In a cross-sectional study of 9-10 year old children in the UK, Jennings, et al. did not find a significant difference in height for children in different quintiles of the Mediterranean Diet Score.[8] Likewise, in a cross-sectional study of Spanish schoolchildren ages 11-12, Arriscado, et al. did not find any difference in height between groups of children with low, medium, and high adherence to a Mediterranean diet according to KIDMED score (p=0.769).[9]

Studies which found an association between Mediterranean-like diet and height:

Several studies did find evidence of a relationship between children's height and adherence to a Mediterranean-like diet. In a cross-sectional study of University students, Štefan, et al. also found a slight difference in height based on tertile of KIDMED score (1.75 m (s.d. 0.09) vs. 1.71 m (s.d. 0.09) for high vs. low adherence, p=0.060).[10] The correlation between height and adherence to a Mediterranean diet was also statistically significant among young women specifically in a subgroup analysis.[10] In a cohort study, Monjardino, et al. also saw a difference in heights between tertiles of KIDMED score among 13-year-old girls (159.9 cm (s.d. 5.7) vs. 158.5 cm (s.d. 5.9) for high vs. low adherence , p=0.007).[11] A cross-sectional study of 10-11 year old boys and girls in Chile also demonstrated a significant difference in height by low, mid, and high adherence based on KIDMED score (139.5 cm (s.d. 6.6) vs. 135.7 cm (s.d. 4.2) for high vs. low adherence , $p<0.0001$).[12]

Summary:

Previous research has not considered the longitudinal effect of a Mediterranean-like diet on peripubertal growth. Some studies, mostly in older adolescents and university students, found an association between cross-sectional height and adherence to a Mediterranean-like diet, while others did not. Thus, this project aimed to address this gap in the literature. We hypothesized that greater adherence to a Mediterranean-like diet would be associated with shorter age-adjusted height at baseline and menarche, and a lower age adjusted growth-rate (prior to menarche).

Methods:

Details about the study population and overall data collection procedures have been outlined in the introductory section. A brief overview and additional information pertinent to this chapter's specific research questions are included below.

In brief, the *Jersey Girl Study* is a longitudinal cohort study based at the Rutgers Cancer Institute of New Jersey (PI: Dr. Elisa Bandera).[13] The sample consists of 202 girls, age 9 or 10 at baseline. Eligibility included residing in New Jersey, living with their biological mothers, no cognitive impairments, the ability to speak and read English, no major medical or surgical conditions known to affect their ability to thrive, stature, amenorrhea, or the timing of puberty; twins were also excluded although siblings were permitted. Data were collected from baseline and follow-up questionnaires filled out by girls' mothers, as well as an initial baseline study appointment, and three 24-hour dietary recalls.

Data collection:

Dietary intake assessment:

Dietary data were collected using three 24-hour recalls conducted by the Dietary Data Entry Center (DDEC) at Cincinnati Children's Hospital Medical Center, as described in detail in the overall methods section. The data were collected on different days of the week, through direct interaction between the DDEC staff and the girls' mothers via telephone.

Assessment of Mediterranean-like diet for this study:

In brief, variations of a Mediterranean Diet Score have been used widely in the pediatric literature. This type of index categorizes food groups as beneficial or detrimental components, and uses a median value of consumption in the study sample for each group as a cutoff to assign a point for levels either above the cutoff, for beneficial components, or below the cutoff, for detrimental components. The total of points is then summed to yield a total score, and categories of this total MDS index can be used for analysis purposes.

Based on the indices used in previous research studies we developed a Mediterranean Diet Score-type index of 'beneficial' and 'detrimental' diet components. Food groups included as 'beneficial' components were vegetables (not potatoes), legumes, nuts, fruits, whole grains, fish, reduced fat/low-fat/fat free dairy, and unsaturated to saturated fat ratio (see details in Table I). Included as a 'detrimental' component was red meat intake. One point was given for an intake above the median intake of the study sample for each beneficial category, or below the median intake for each detrimental category, and the points were summed to give a total score which ranges from 0 to 9. The specific types of foods included in each category are detailed in Table I of the introductory section.

Measurement of Height:

Trained members of the *Jersey Girl Study* staff recorded girls' standing and sitting heights (to the nearest 0.1 cm) at the baseline study visit. Girls' heights for each annual follow-up (until menarche) were collected using forms mailed to participants and filled out by mothers and/or girls. Height at menarche for the purposes of this analysis was defined as the height reported during the same annual follow-up where the girl's first period was reported.

Statistical analyses:

We calculated summary statistics (counts and percent for categorical variables, and mean or median and standard deviation for continuous variables) for the distribution of height at baseline, height at menarche, and covariates, for all girls, and for those who had high vs. medium vs. low adherence to a Mediterranean-like diet (p-values for differences were computed using chi-square tests, ANOVA, and Brown-Mood tests). Separate linear regression models were fit to compare predicted mean height at baseline and height at menarche based on girls' adherence to a Mediterranean-like diet. Linear regression models were also fit to compare predicted mean height-for-age z-score at baseline and predicted mean height-for-age z-score at menarche, based on girls' adherence to a Mediterranean-like diet. A linear mixed-effects growth model (using an uncorrelated covariance structure) was fit for the height data to examine the relationship between peripubertal growth trajectory and adherence to a Mediterranean-like diet. Subject-specific random effects must be included in the model, particularly because girls have different numbers of height measurements that were taken at different times and differently spaced. A similar mixed-effects growth model was also fit using the height-for-age z-score data to examine growth trajectory by adherence to a Mediterranean-like diet.

Potential confounders of interest that were considered for adjustment in the multivariable models include: age at baseline (continuous; where appropriate), race (white or other), annual household income (< \$70,000, \$70,000-\$84,999, \$85,000-\$99,999, > \$100,000), mother's education (high school-associate degree, bachelor's degree, master's degree, professional/doctoral degree), total energy intake, girl's BMI category at baseline, and total hours of girl's physical activity per week. As there is a hereditary component to height, parental adult height, was adjusted for using an adjusted midparental height calculated based on the literature (father's height minus 13 cm, plus the mother's height, divided by two; this was converted to inches since that was the unit of analysis for height.)[14] Model covariates were selected based on a priori hypotheses, as well as statistical significance levels in the models.

Results:

Height at baseline and height at menarche for girls in the study sample, as well as the distribution of covariates in the models, are reported in Table 3.1. Girls in the study had a mean baseline height of 54.92 inches (3.17 s.d.) and a mean height at menarche of 62.86 inches (3.03 s.d.). Baseline height and height at menarche were not different across Mediterranean-like diet adherence groups.

The results of linear regression models for mean height at baseline and height at menarche are presented in table 3.2a. In the full model adjusted for age at baseline, race, midparental height, and total energy intake at baseline, no significant association was seen between mean height at baseline and diet adherence (predicted mean height 55.62 (54.85-56.39) inches for low adherence vs. 55.24 (54.44-56.05) inches for high adherence; change 0.054 (-0.15-0.26) inches per unit increase in diet adherence score). In the full model (adjusted for age at baseline and midparental height), no association was seen between mean height at menarche and diet adherence (predicted mean height 62.99 (62.35-63.62) inches for low adherence vs. 62.94 (62.06-63.83) inches for high adherence; change -0.04 (-0.28-0.19) inches per unit increase in diet adherence score). Similar linear regression models for mean height at baseline and height at menarche, using height-for-age z-scores, are presented in table 3.2b. The results from crude and fully adjusted models likewise show no significant relationship between height z-scores and Mediterranean-like diet adherence.

The results of linear mixed-effects models for the effect of Mediterranean-like diet adherence on growth over time (prior to menarche) are presented in table 3.3a. In the crude and adjusted models, the interaction between age and diet adherence showed no significant association with height (estimate -0.07 (95% CI-0.27-0.13) for the high adherence group in the full model adjusted for race, midparental height, BMI status at baseline, and total energy intake at baseline). Mixed models run using height for age z-scores (results presented in table 3.3b), also did not show a significant interaction between age and diet adherence.

Discussion:

The results of this study did not show any evidence of an association between Mediterranean-like diet adherence on either a categorical or continuous scale, and height at baseline or menarche (either height or height-for-age z-score), in our sample of peripubertal girls. Our mixed-effects models for height and height z-score also did not show statistically significant differences in height growth rate prior to menarche in girls based on adherence to a Mediterranean-like diet.

The failure to identify any association between Mediterranean-like diet adherence and cross-sectional height concurs with several previous studies. Cross-sectional studies in 6-8 year old girls,[7] 9-10 year-old children,[8] and 11-12 year-old children[9] did not demonstrate a significant relationship between a Mediterranean diet and height. Conversely, several other previous studies did find an association between diet adherence and cross-sectional height, in the opposite direction of what we hypothesized for this study. A cross-sectional study of University students found a difference in height based on KIDMED score (1.75 m (s.d. 0.09) vs. 1.71 m (s.d. 0.09) for high vs. low adherence, $p=0.060$).[10] Another cohort study also saw a difference in height depending on KIDMED score among 13-year-old girls (159.9 cm (s.d. 5.7) vs. 158.5 cm (s.d. 5.9) for high vs. low adherence , $p=0.007$).[11] A cross-sectional study of 10-11 year old children also demonstrated a difference in height by adherence based on KIDMED score (139.5 cm (s.d. 6.6) vs. 135.7 cm (s.d. 4.2) for high vs. low adherence, $p<0.0001$).[12]

There could be several potential reasons that we did not see a difference as seen in these studies. There may truly be no association between Mediterranean-like diet adherence and height, it may not exist in our population, or limitations in our analysis (discussed further below) may have prevented us from identifying these differences. Since height was not a primary outcome in these previous studies, the evidence was mostly from unadjusted statistical tests, which did not account for important measured and unmeasured confounders, such as race, BMI, midparental height, energy intake, environmental factors, and lifestyle factors. These factors could also differ across populations, and none of these studies took place in a U.S. population. The foods and nutrients consistent with a Mediterranean-like diet in other international populations may differ, and moreover, all of the studies which identified an association based their diet adherence on the KIDMED index. The KIDMED index includes not only information on traditional components of a Mediterranean-like diet, but also asks about sweet and candy consumption, fast food intake, and breakfast consumption and composition. Thus, using KIDMED score as the measure of diet adherence may result in different associations than using an alternative measure of diet adherence. Also, our time points for measurement, at baseline when over half of girls had already begun puberty, and around menarche which is still likely a year or more away from final adult height, may not have represented the most meaningful points to see a difference in growth based on diet. Ours was the first longitudinal study to investigate the relationship between a Mediterranean-like diet and measurements of growth in children/adolescents over time. Our mixed-effects growth models did not show any evidence that level of adherence to a Mediterranean-like diet modified growth rate during puberty.

Several limitations of our study should be acknowledged, which may explain why we did not observe any associations with height at baseline or menarche. First, the sample was predominantly White, with highly educated mothers, which may limit the generalizability of the findings to other populations. The sample size was relatively small (n=200), which may have limited the statistical power to detect small differences in outcomes. It is likely that growth over the entire pubertal period can be best estimated with a nonlinear model; however, we were constrained in our ability to model by the number of height observations per girl – due to timing of menarche (as well as missing data), 50% of girls had 3 or fewer height observations. There is an inherent risk in using self-reported height data for inaccuracy and overestimation of height, though it is unlikely this bias would have been differential based on girls' diet adherence. We collected dietary data only at baseline and assumed that dietary patterns did not change over the follow-up period.

However, our study did have several strengths, including that height at baseline was measured accurately and consistently by research staff for all girls in the study. The dietary recall interviews allowed for capturing intake of exact foods consumed, and represented both weekday and weekend consumption. The study questionnaire and anthropometric measurements taken also provided us with a wide range of potentially relevant confounding variables for adjustment in our regression models.

The results of this study contribute to a growing body of research on the topic of a Mediterranean-like diet and growth in peripubertal girls. Studies specifically examining height and growth as outcomes are very limited, and while a few studies have found some association between height and adherence to a Mediterranean-like diet, further research is still needed in both U.S. and international populations.

Table 3.1: Height and Selected Covariates, Overall and According to Mediterranean-like Diet Adherence

	Total (n=202)	**Low MD Adherence (MDS 0-3) (n=72, 35.64%)**	**Med. MD Adherence (MDS 4-5) (n=85, 42.08%)**	**High MD Adherence (MDS 6-9) (n= 45, 22.28%)**	**P-value**
Child Height at Baseline, in. (mean (SD))	54.92 (3.17)	55.00(3.16)	54.83 (3.28)	54.97 (3.02)	0.94
Child Height at Baseline, in. (median)	54.86	54.94	54.37	54.96	0.91
Height at menarche, in. (mean (SD))	62.86 (3.03)	62.82 (2.74)	62.84 (3.15)	63.01 (3.41)	0.96
Height at menarche, in. (median)	62.50	62.00	62.50	63.13	0.73
Midparental height, in. (mean (SD))	64.70 (2.34)	64.60 (2.41)	64.62 (2.19)	65.01 (2.51)	0.61
Midparental height, in. (median)	64.44	63.94	64.44	65.19	0.73
Child age at baseline (mean (SD))	10.02 (0.58)	10.09 (0.63)	9.97 (0.54)	9.97 (0.54)	0.36
Race, n (%)					0.09
White	177 (87.62)	65 (90.28)	76 (89.41)	36 (80.00)	
Other	22 (10.89)	6 (8.33)	7 (8.24)	9 (20.00)	
Total energy intake, kcal (mean (SD))	1718.77 (384.77)	1625.02 (369.62)	1773.74 (385.96)	1764.94 (382.80)	**0.03**
Child BMI, kg/m^2 (mean (SD))	18.04 (3.10)	17.80 (3.27)	18.28 (3.01)	17.98 (3.01)	0.63
Child BMI category (based on percentile for age), n (%)					0.36
Underweight	12 (5.94)	7 (9.72)	2 (2.35)	3 (6.67)	
Healthy weight	147 (72.77)	49 (68.06)	64 (75.29)	34 (75.56)	
Overweight/obese	43 (21.29)	16 (22.22)	19 (22.35)	8 (17.78)	

Categories may not total to 100% due to missing values;
p-values are from ANOVA for means, Brown-Mood test for medians, and chi-square for categories

Table 3.2a: Mean Height at Baseline (in) and Height at Menarche (in) and 95% Confidence Intervals According to Mediterranean-like Diet Adherence, Estimated from Multiple Linear Regression as Described Below

	Mean Height at Baseline (in) (95% confidence interval)		**Mean Height at Menarche (in) (95% confidence interval)**	
	Model adjusted only for age	**Fully adjusted model***	**Model adjusted only for age at baseline**	**Fully adjusted model***
Adherence to a Mediterranean-like diet				
Low adherence (0-3)	54.83 (54.15-55.50)	55.62 (54.85-56.39)	62.84 (62.07-63.61)	62.99 (62.35-63.62)
Medium adherence (4-5)	54.93 (54.31-55.55)	55.53 (54.82-56.25)	62.84 (62.10-63.57)	62.75 (62.16-63.34)
High adherence (6-9)	55.06 (54.21-55.91)	55.24 (54.44-56.05)	62.98 (61.87-64.08)	62.94 (62.06-63.83)
Change per unit change in adherence to a Mediterranean-like diet (continuous)	0.14 (-0.09-0.37)	0.054 (-0.15-0.26)	-0.049 (-0.33-0.24)	-0.04 (-0.28-0.19)

*Full model for height at baseline is adjusted for age at baseline, race, midparental height, and total energy intake

*Full model for height at menarche is adjusted for age at baseline and midparental height

Table 3.2b: Mean Height for Age Z-score at Baseline and Height for Age Z-score at Menarche and 95% Confidence Intervals According to Mediterranean-like diet Adherence, Estimated from Multiple Linear Regression as Described Below

	Mean Height for Age Z-score at Baseline (95% confidence interval)		**Mean Height for Age Z-score at Menarche (95% confidence interval)**	
	Crude model	**Fully adjusted model***	**Crude model**	**Fully adjusted model***
Adherence to a Mediterranean-like diet				
Low adherence (0-3)	0.13 (-0.11-0.38)	0.42 (0.14-0.71)	0.33 (0.05-0.62)	0.75 (0.43-1.07)
Medium adherence (4-5)	0.17 (-0.05-0.40)	0.38 (0.11-0.65)	0.47 (0.20-0.75)	0.71 (0.40-1.02)
High adherence (6-9)	0.23 (-0.09-0.54)	0.28 (-0.02-0.58)	0.45 (0.03-0.86)	0.64 (0.28-0.99)
Change per unit change in adherence to a Mediterranean-like diet (continuous)	0.053 (-0.32-0.14)	0.02 (-0.06-0.09)	0.036 (-0.069-0.14)	0.013 (-0.07-0.096)

*Full model for height for age z-score at baseline is adjusted for midparental height, race, and total energy intake at baseline

*Full model for height for age z-score at menarche is adjusted for midparental height, race, and total energy intake at baseline

Table 3.3a: Mixed-effects Model Results for Growth Rate (Prior to Menarche) Related to Mediterranean-like Diet Adherence

	Estimate (95% C.I.)	
	Crude model	**Fully adjusted model**
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	Ref.	Ref.
Medium adherence (4-5)	0.09 (-0.09-0.26)	0.08 (-0.10-0.26)
High adherence (6-9)	-0.07 (-0.27-0.13)	-0.07 (-0.28-0.13)
Adherence to a Mediterranean-like diet (continuous)	-0.02 (-0.06-0.03)	-0.02 (-0.09-0.03)
*Full model is adjusted for race, midparental height, BMI category at baseline, and total energy intake at baseline		

Table 3.3b: Mixed-effects Model Results for Height (z-score) Related to Mediterranean-like Diet Adherence

	Estimate (95% C.I.)	
	Crude model	**Fully adjusted model**
Adherence to a Mediterranean-like diet		
Low adherence (0-3)	Ref.	Ref.
Medium adherence (4-5)	0.02 (-0.03-0.07)	0.009 (-0.04-0.06)
High adherence (6-9)	0.02 (-0.04-0.07)	0.01 (-0.04-0.07)
Adherence to a Mediterranean-like diet (continuous)	-0.0005 (-0..01-0.01)	-0.0001 (-0.01-0.01)
*Full model is adjusted for race, midparental height, BMI category at baseline, and total energy intake at baseline		

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Overall Conclusion and Summary:

Although timing and tempo of puberty varies among individuals, there has been an observed population-level trend toward earlier puberty, which presents psychological as well as clinical risks to girls. Body composition, fat distribution, and obesity in the peripubertal period are also risk factors for certain adult health problems, including adult obesity and breast cancer. Diet is one of the primary and most easily modifiable factors that can influence body composition and measurements, as well as puberty, in girls. Over the years, the field of nutritional epidemiology has evolved from looking at individual foods and nutrients to taking a more holistic approach by evaluating dietary patterns, and the Mediterranean diet in particular has received attention. As girls do not consume specific foods and nutrients in isolation from one another, research on dietary pattern as a whole is more realistic, and changes in overall dietary pattern are more practical to implement than focusing on certain foods. However, research into the role of a Mediterranean-like diet on pubertal development in U.S. girls is limited. A thorough review of the current literature showed that research into the effects of diet on puberty timing and outcomes has mainly consisted of studying specific foods and nutrients, and a Mediterranean-like dietary pattern has not been researched with regard to these outcomes. While the associations of a Mediterranean-like diet with body fatness have been studied previously, the evidence in children has been inconclusive, and only one previous study has been performed in a healthy pediatric population in the U.S. Moreover, few studies have data on the relationship between a Mediterranean-like diet and height in children throughout the pubertal period, and none have looked at longitudinal growth.

To our knowledge, our study is the first to explore puberty timing, outcomes, and growth in a pediatric population in the context of adherence to a Mediterranean-like diet. It is also one of only a few studies in the U.S. to examine the effects of a Mediterranean-like diet on BMI and obesity in children. To summarize, our study found that high adherence to a Mediterranean-like diet, when compared to low adherence, was associated with a significantly reduced odds of breast development at age 9 or 10. Further analysis suggested that this association may have been driven by girls' consumption of fish and reduced/low/non-fat dairy, in particular. Our models also suggested that there was a corresponding trend toward later age at thelarche with higher Mediterranean-like diet adherence. Similarly, high adherence to a Mediterranean-like diet (compared to low adherence), as well as diet adherence on a continuous scale, were associated with greater time to menarche. Our additional models indicated that this may have been driven by higher consumption of vegetables and reduced/low/non-fat dairy. These results were consistent with previous findings that components of a Mediterranean-like diet, such as higher intakes of monounsaturated fats and fiber, and lower intakes of red meat, were associated with a later age at menarche, and that consumption of fiber was associated with later thelarche. However, most of the specific food group components of our diet index did not show a significant association with puberty outcomes. Previously observed associations should be considered in the context of overall dietary patterns, such as a Mediterranean-like diet, which are consistent with the intakes of the food groups studied. Height at the start of the study, height at menarche, and growth rate during puberty did not differ according to Mediterranean-like diet adherence, according to our models. Although BMI was positively associated with the prevalence of puberty outcomes in our models, no interaction effect of BMI with diet adherence was seen in our study. Furthermore, though we hypothesized that a Mediterranean-like diet would show associations with BMI and body fatness, our results did not demonstrate any significant relationship with BMI, obesity, or measures of body composition and fat distribution, in this population.

While our study should be interpreted in the context of its limitations, such as its relatively small and homogeneous sample (White, high in income and education) and the data being self-reported, our findings represent an important contribution to the literature on the relationship of a Mediterranean-like dietary pattern with growth and puberty outcomes. Our detailed collection of dietary recalls and prospective data collection on puberty outcomes and growth over the pubertal period, in addition to data on confounders collected in our questionnaire, was likely more accurate than retrospective, cross-sectional, and food frequency questionnaire data used in many other studies. We also used a purer diet adherence index, which did not include questions on extraneous factors such as fast food and breakfast consumption, as the popular KIDMED score does. Further research is necessary to confirm our findings in other and more heterogeneous U.S. pediatric populations, especially since the foods and nutrients consistent with a more Mediterranean-like diet in this country may be different from those in others where previous research has been performed. Finally, a next step for this research would be investigating the mechanism through which Mediterranean-like diet may be influencing puberty and growth by measuring girls' sex-hormone binding globulin, estrogen, androgen, and gonadotropin levels in relation to their diet adherence status.

Our results suggest that consuming a more Mediterranean-like diet, high in plant-based foods (like fiber-rich whole grains, vegetables, fruits, nuts, and legumes), unsaturated fats, and fish, and low in red and processed meats, may decrease girls' risk of earlier puberty. Delaying puberty could consequently lead to decreased risk of adverse psychological, behavioral, and clinical outcomes in these girls, including low self-esteem, drug abuse, polycystic ovarian syndrome, and breast cancer risk. Half of the girls in our study had reached thelarche by age 10, and previous research has shown a relationship between thelarche at age 10 or earlier and a 20% higher risk of breast cancer;[1] thus, delaying thelarche could have a significant impact on the health of peripubertal girls such as those in our sample. Adopting an overall healthier dietary pattern, such as a Mediterranean-like diet, may be easier for girls and their families than changing the intake of particular foods, and current average daily intake of vegetables, fruits, legumes, dairy, whole grains, seafood, and nuts in this age group are currently well below the recommended daily intake.[2] Consumption of a Mediterranean-like diet is consistent with existing recommendations for children in the U.S., including replacing high-calorie snacks with natural fruits and nuts free of added sugars and salt, replacing refined grains with whole grains, and choosing oils rather than solid fats.[2] In conclusion, our findings provide further support for existing public health efforts that encourage following a healthy and balanced diet such as a Mediterranean-like dietary pattern.

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Appendix A: Supplemental Tables

Supplemental table 1a: Poisson Regression Analysis for Thelarche at Baseline (Yes vs. No), Related to Mediterranean-like Diet Adherence by Component

	Prevalence Ratio (95% confidence interval)		
Mediterranean-like dietary component	**Models adjusted only for age**	**Fully adjusted models (for covariates but not mutually adjusted for other components)***	**Fully and mutually adjusted model (adjusted for all other components)***
Red meat (low vs. high consumption)	0.92 (0.74-1.15)	1.07 (0.86-1.32)	1.14 (0.92-1.40)
Fish (consumers vs. nonconsumers)	0.81 (0.61-1.08)	0.79 (0.62-1.01)	**0.77 (0.61-0.97)** ǂ
Fruit (high vs. low consumption)	1.08 (0.87-1.35)	0.96 (0.78-1.18)	0.93 (0.75-1.16)
Fiber-rich whole grains (high vs. low consumption)	0.96 (0.76-1.19)	0.89 (0.73-1.10)	0.89 (0.73-1.10)
Unsaturated:saturated fat ratio (high vs. low consumption)	1.18 (0.95-1.48)	0.95 (0.77-1.18)	0.89 (0.73-1.10)
Dairy (high vs. low consumption)	0.86 (0.69-1.08)	**0.76 (0.63-0.93)** §	**0.75 (0.60-0.93)** §
Nuts (consumers vs. nonconsumers)	0.90 (0.72-1.12)	0.85 (0.69-1.05)	0.90 (0.73-1.13)
Legumes (consumers vs. nonconsumers)	1.01 (0.81-1.27)	1.06 (0.86-1.31)	1.12 (0.92-1.38)
Vegetables (high vs. low consumption)	1.12 (0.90-1.40)	1.02 (0.83-1.26)	1.09 (0.88-1.34)

*Full model is adjusted for age at baseline, girl's total energy intake, girl's BMI z-score at baseline, and mother's age at breast development

§ $p<0.01$, ǂ $p<0.05$

Cutpoints for consumption (based on medians): 40.41 g/day for red meat, 177 g/day for fruits, 16.5 g/day for fiber-rich whole grains, 1.53 for fat ratio, 214.4 g/day for low- and no-fat dairy, & 90.58 g/day for vegetables. Fish, nut, & legume consumption was categorized as consumers vs. nonconsumers due to a median average daily consumption of 0 g in our sample.

Supplemental table 1b: Logistic Regression Analysis for Thelarche at Baseline (Yes vs. No), Related to Mediterranean-like Diet Adherence by Component

	Odds Ratio (95% confidence interval)		
Mediterranean-like dietary component	**Models adjusted only for age**	**Fully adjusted models (for covariates but not mutually adjusted for other components)***	**Fully and mutually adjusted model (adjusted for all other components)***
Red meat (low vs. high consumption)	0.78 (0.43-1.40)	0.99 (0.50-1.96)	1.24 (0.60-2.57)
Fish (consumers vs. nonconsumers)	0.60 (0.31-1.14)	0.51 (0.24-1.10)	**0.42 (0.19-0.96)** ǂ
Fruit (high vs. low consumption)	1.22 (0.68-2.18)	0.83 (0.42-1.64)	0.66 (0.31-1.39)
Fiber-rich whole grains (high vs. low consumption)	0.87 (0.48-1.55)	0.64 (0.33-1.27)	0.57 (0.28-1.18)
Unsaturated:saturated fat ratio (high vs. low consumption)	1.53 (0.85-2.77)	0.83 (0.41-1.68)	0.69 (0.32-1.49)
Dairy (high vs. low consumption)	0.68 (0.38-1.22)	**0.41 (0.20-0.84)** ǂ	**0.37 (0.17-0.80)** ǂ
Nuts (consumers vs. nonconsumers)	0.74 (0.42-1.33)	0.55 (0.28-1.10)	0.64 (0.31-1.31)
Legumes (consumers vs. nonconsumers)	0.99 (0.54-1.80)	1.17 (0.59-2.32)	1.34 (0.64-2.81)
Vegetables (high vs. low consumption)	1.34 (0.75-2.40)	1.15 (0.59-2.26)	1.46 (0.70-3.05)

*Full model is adjusted for age at baseline, girl's total energy intake, girl's BMI z-score at baseline, and mother's age at breast development

ǂ $p<0.05$

Cutpoints for consumption (based on medians): 40.41 g/day for red meat, 177 g/day for fruits, 16.5 g/day for fiber-rich whole grains, 1.53 for fat ratio, 214.4 g/day for low- and no-fat dairy, & 90.58 g/day for vegetables. Fish, nut, & legume consumption was categorized as consumers vs. nonconsumers due to a median average daily consumption of 0 g in our sample.

Supplemental Table 1c: Hazard Ratios and 95% Confidence Intervals for Time to Menarche (on an age time-scale) from a Cox Proportional Hazards Model, According to Mediterranean-like diet Adherence by Component

	Hazard Ratio (95% confidence interval)		
Mediterranean-like dietary component	**Models adjusted only for age**	**Fully adjusted models (for covariates but not mutually adjusted for other components)***	**Fully and mutually adjusted model (adjusted for all other components)***
Red meat (low vs. high consumption)	1.01 (0.74-1.36)	1.15 (0.83-1.60)	1.21 (0.86-1.71)
Fish (consumers vs. nonconsumers)	0.89 (0.63-1.25)	0.71 (0.49-1.03)	0.79 (0.53-1.18)
Fruit (high vs. low consumption)	0.93 (0.69-1.26)	0.80 (0.57-1.13)	0.80 (0.56-1.15)
Fiber-rich whole grains (high vs. low consumption)	1.09 (0.81-1.48)	1.02 (0.74-1.41)	1.08 (0.77-1.52)
Unsaturated:saturated fat ratio (high vs. low consumption)	1.18 (0.86-1.61)	0.92 (0.65-1.31)	0.93 (0.64-1.36)
Dairy (high vs. low consumption)	0.87 (0.65-1.19)	**0.65 (0.46-0.92)** ǂ	**0.63 (0.43-0.91)** ǂ
Nuts (consumers vs. nonconsumers)	1.09 (0.80-1.48)	0.94 (0.68-1.31)	1.02 (0.72-1.44)
Legumes (consumers vs. nonconsumers)	1.00 (0.73-1.37)	0.88 (0.62-1.23)	1.04 (0.72-1.51)
Vegetables (high vs. low consumption)	**0.72 (0.53-0.98)** ǂ	**0.57 (0.41-0.79)** §	**0.66 (0.46-0.95)** ǂ

*Full model is adjusted for age at baseline, race, total energy intake, physical activity, girl's BMI z-score at baseline, and maternal age at menarche

§ p<0.01, ǂ p<0.05

Cutpoints for consumption (based on medians): 40.41 g/day for red meat, 177 g/day for fruits, 16.5 g/day for fiber-rich whole grains, 1.53 for fat ratio, 214.4 g/day for low- and no-fat dairy, & 90.58 g/day for vegetables. Fish, nut, & legume consumption was categorized as consumers vs. nonconsumers due to a median average daily consumption of 0 g in our sample.

Appendix B: **Previous Studies Reporting on the Association Between Mediterranean-like Diet and BMI/Obesity**

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
Grammatikopoulou, et al.[1]	2018	Cross-sectional study in Greece	157 boys and 162 girls	10-18	KIDMED	BMI, WHO definition of overweight/obesity	Kruskal-Wallis test, multinomial logistic regression	Mean KIDMED score differed by BMI status (p=0.049); low MD adherence was associated with obesity (compared to high adherence) OR=4.2, p=0.005 in adjusted model; overweight status was not associated with KIDMED score	Age, gender, residence status
Bacopoulou, et al.[2]	2017	School-based interventional study in Greece	1610	12-17	KIDMED	BMI, waist circumference (WC), waist-to-height-ratio (WHtR)	t-test/one-way ANOVA, using Bonferroni correction	WC decreased (71.1±9.2 cm to 70 .6 ± 9.0 cm) as KIDMED score increased (p = 0.020)	-
Muros, et al.[3]	2017	Cross-sectional study in Chile	515	10 & 11	KIDMED	Weight, BMI, skinfolds, WC	U-test, Kruskal-Wallis test	MD adherence differed by BMI, percent body fat, and WC (p<0.0001); MD adherence was consistently negatively associated with percent BF (r= -0.302), subscapular skinfold thickness (r=-0.329), WC (r=	-

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
								-0.231), BMI (r= -0.286)	
osi, et al.[4]	2017	Cross-sectional study of children in Parma, Italy	690	9-11	KIDMED	BMI groups	Pearson chi-square	No association was found between BMI group and KIDMED score	-
Štefan, et al.[5]	2017	Cross-sectional study of university students in Croatia	198		KIDMED	BMI	ANOVA	Negative relationship between KIDMED score and BMI (p<0.001) and percent fat mass (p<0.001)	-
Novak, et al. [6]	2017	Cross-sectional study of adolescents from Lithuania and Serbia	3071	14-18	KIDMED	BMI status	regression models	Poor adherence was associated with being overweight/obese (OR 0.74, 0.59-0.92)	Gender, self-rated health, SES, psychological distress, physical activity, sedentary behavior
Mistretta, et al.[7]	2016	Cross-sectional study in Sicily	1643	11-16	KIDMED score	BMI, WC, fat mass (FM)	regression models	High adherence to MD associated with lower odds of overweight/obese (OR 0.66 95% CI 0.47-0.93 in girls); inverse relationship between KIDMED score and BMI (β= -0.063 for girls), WC (β= -0.011 for girls), FM (β= -0.028 for girls)	gender, age, SES, BMI, physical activities, energy intake, blood pressure, parents' education, parents' occupation
Martin-Calvo, et	2016	Prospective	6,002	8-15	KIDMED	BMI change	used GEE	A 2 pt. increment in	sex, starting

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
al.[8]		U.S. study(Growing up Today Study II)	females and 4,916 males				with repeated measures within subjects to assess association between MDP & BMI change	KidMed was associated with a lower gain in BMI (−0.04 kg/m2; p=0.001; -0.03 kg/m2, p=0.06 for girls only); greater increase in adherence to KIDMED was related to a lower gain in BMI in both the concurrent (p-for-trend<0.001) and the subsequent period (p-for-trend=0.002)	BMI, physical activity, sedentary time, time of follow-up; baseline KIDMED where applicable; BMI change in previous interval where applicable
Martino, et al.[9]	2016	Cross-sectional study in Italy, Calabrian Sierras Community Study (CSCS)	863 boys and 780 girls	6-14	Modified KIDMED	Metabolic syndrome (MS)	t-tests, chi-square, ANOVA	Prevalence of MS was higher in the low adherence MD group (OR: 1.8; 95% C.I.: 1.06–3.11; p = 0.013)	-
Cakir, et al.[10]	2016	Cross-sectional study of children	181	11-12?	KIDMED	BMI	Correlation	KIDMED score was negatively correlated with BMI (r=-0.53, p<0.05)	-
Ferranti, et al.[11]	2016	Cross-sectional study of students in Sicily	1586	11-14	KIDMED	BMI, FM, WC	Chi-square	Adherence to MD was higher in underweight/normal weight adolescents (p=0.003)	-
Zhong, et al.[12]	2016	Participants from	793 at baseline	< 20	Modified KIDMED	Obesity, WC	ANOVA, Kruskal-	MD was not associated with	Age, sex, race, diabetes,

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
		SEARCH nutrition ancillary cohort study of youth with diabetes					Wallis, chi-square, linear adjusted and mixed models	obesity- (p=0.75 for BMI z-score and p=0.83 for waist circumference); p-values from adjusted cross-sectional and longitudinal models were also nonsignificant	parental education, family income, family history of diabetes, site, physical activity, sedentary behavior, smoking, total calories, insulin regimen, daily insulin dose, HbA1c
Eloranta, et al.[13]	2016	Cross-sectional study of Finnish children	204 boys and 198 girls	6-8	MDS	BMI, WC	Linear regression	MDS showed no association with BMI (p=0.858 in girls) or waist circumference (p=0.535 in girls)	Age, physical activity, electronic media time, household income
Voltas, et al.[14]	2016	Cohort study of adolescents in Spain	241	12-15	Krece plus	BMI	Chi-square, regression models	BMI category during phases 1 and 3 was not significantly associated with MD adherence (p>0.5)	Age, gender, SES
Papadaki, et al. [15]	2015	Cross-sectional school-based study in Greece	525	12-18	KIDMED	BMI	Chi-square, t-test, linear regression	No correlation found with BMI (p>0.05)	-
Tognon, et al.[16]	2014	IDEFICS cohort study in 8 European countries	16220, follow-up on 9114	2-9	FFQ based MDS	BMI, WC, waist-to-height-ratio (WHtR), FM, skinfolds	Logistic and linear regression models	High MDS inversely associated with overweight including obesity	age, sex, SES (parental education & income), study

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
								(OR 0.85, 95% CI: 0.77-0.94) and percent FM (β =-0.22, 95% CI: -0.43, -0.01); High baseline MDS 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 WHtR (OR = 0.88, 95% CI: 0.78-0.99) with a similar trend observed for % FM (p = 0.06)	center & physical activity; baseline age- and sex-specific BMI z-scores
Velasquez-Lopez, et al.[17]	2014	Intervention study in Mexico		Median age 11	Diet intervention	BMI, FM, weight, height, fat pct., lean mass, WC, hip circumference	Paired t-tests	MD group had decreased BMI (p=0.001), FM (p=0.001)	-
Roccaldo, et al.[18]	2014	Cross-sectional study in Italy	1740	8-9	KIDMED	BMI	Chi-square	MD adherence rates did not differ significantly with BMI	-
Santomauro, et al.[19]	2014	Cross-sectional study of students in Florence Italy	1127	14-20	KIDMED	BMI status	Multiple logistic regression	Being normal weight, overweight, or obese were significantly protective (OR 0.41 (95% CI 0.25-0.68); OR 0.47 (95%CI 0.24-0.94)) against poor MD adherence	-

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
Noale, et al.[20]	2014	Cross-sectional survey of Italian adolescents	565	12-19	MDS	BMI	Linear models, u-tests or Kruskal-Wallis test	No significant association between MD adherence and BMI ($p=0.702$)	-
Arriscado, et al.[21]	2014	Cross-sectional study of Spanish school children	321	11-12	KIDMED	BMI, WC, HC, pct. FM, IOTF definition of obesity	t-tests, ANOVA, u-tests, Kruskal-Wallis	No association between adherence and BMI, obesity, WC, HC, or pct. BF (p all > 0.1)	-
Navarro-Solera, et al.[22]	2014	Cross-sectional study of schoolchildren in Valencia, Spain	777	8-16	KIDMED	BMI, WHO definition of obesity		Significant association between adherence to MD and overweight/obesity in the total sample and in females ($p<0.05$ for both)	-
Antonogeorgos, et al.[23]	2013	Cross-sectional study of Greek children (PANACEA study)	529 boys and 596 girls	10-12	KIDMED	BMI	Multiple logistic regression	MD adherence inversely associated with obesity status only in families in which at least one parent was of high educational level (stratum-specific adjusted odds ratio: 0.41; 95% CI 0.17–0.98), but not those in which both parents were of low educational level	Age, gender, county (rural/urban), activity, breastfeeding history, breakfast consumption, meal skipping, parent obesity
Grosso, et al.[24]	2013	Cross-sectional study	1135	13-16	KIDMED	BMI, IOTF definition of	Chi-square, t-tests, U-	Obesity associated with decreased odds	Residence, SES, gender,

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
		of Italian adolescents in Sicily				obesity	tests, ANOVA, Kruskal-Wallis, logistic regression	of high adherence (OR 0.59, 95% CI 0.37-0.94) (also significant for overweight)	physical activity
Grao-Cruces, et al.[25]	2013	Cross-sectional study of adolescents in southern Spain	1973	11-18	KIDMED	BMI, percent body fat		No significant association between MD adherence and BMI score or percent body fat	-
Lydakis, et al.[26]	2012	Cross-sectional study of Greek children	277	12	KIDMED	Weight, Height, WC, BMI	t-tests, ANOVA, multiple linear regression models	Overweight and obese children had significantly lower KIDMED score in comparison to children with normal body mass index (BMI) p=0.044 from ANOVA (Same trend seen with WC)	-
Monjardino, et al.[27]	2012	EPITeen cohort of Portuguese adolescents	1180	13, 17	KIDMED	BMI	ANOVA, Kruskal-Wallis	No significant association between MD and BMI at baseline or 4-year follow-up (p=0.566 in girls at baseline)	-
Farajian, et al.[28]	2011	Cross-sectional study of Greek schoolchildren	4786	10-12	KIDMED based on FFQ	BMI, WC, waist-to-hip ratio (WHR), WHtR, percent body fat (BF)	ANOVA, chi-square, t-tests	No difference in KIDMED score between overweight and normal children; no difference in any of the anthropometric	-

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
								measurements by KIDMED score category	
Jennings, et al.[29]	2011	Cross-sectional study of British children	1700	9-10	MDS	WC, percent BF, BMI z-scores, WHtR	ANCOVA regression models	No association between MD score and weight status	gender, parental education, underreporting, energy density, physical activity
Arvaniti, et al.[30]	2011	Cross-sectional study of children from the Athens area (PANACEA study)	700	10-12	KIDMED	BMI, IOTF definition of obesity	Chi-square, t-test; logistic regression	No significant association between MD and BMI (p=0.74; OR from logistic regression 1.02 (95% CI 0.97-1.08)) or overweight/obese (p=0.67)	age, sex, physical activity, energy
Mazaraki, et al.[31]	2011	Cohort study of Greek adolescents	365	12-17	KIDMED	BMI, WC, HC, IOTF definition of obesity	Pearson correlations	KIDMED score positively related to BMI (r=0.122, p=0.02), and WC (r=0.118, p=0.02)	-
Magriplis, et al.[32]	2011	Cross-sectional population based study (GRECO study)	4580	10-12	KIDMED	BMI, percent body fat, FM, IOTF definition of obesity	Chi-square or U-test	No significant association between MD adherence and BMI (p=0.311) , percent body fat, (p=0.193) obesity (p=0.348)	-
Perez-Gallardo, et al.[33]	2011	Cross-sectional study of Spanish schoolchildren	350	6-9	KIDMED	BMI	Correlation	Negative correlation between adherence and BMI (r=-0.133; p< 0.05)	-

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
Schroeder, et al.[34]	2010	Cross-sectional study in Spain	2513	10-24	KIDMED and 24 h recall	WC, WHtR	Linear models	5-point increase in KIDMED associated with mean decline of 1.54 cm in sex-, age- and height-adjusted WC	Age, sex, energy intake, leisure time physical activity, low energy reporting, maternal education
Lazarou, et al.[35]	2010	National cross-sectional study in Cyprus, CYKIDS study	1140	Mean age 10.7	KIDMED	BMI	Logistic regression	Inverse association between KIDMED and overweight/obese (80% less likely to be ow/ob; 95% confidence interval 0.041–0.976), but disappeared after adjustment for physical activity (OR 0.20, 95% CI 0.021–1.86)	Age, gender, parental obesity status, parental education level, physical activity, dietary beliefs & behaviors
Kontogianni, et al.[36]	2008	Cross-sectional study in Greece	1305	3-18	KIDMED score	BMI	Partial correlations	Higher KIDMED score associated with lower BMI (r = -0.092; p = 0.031),	age, sex, time spent in sedentary & physical activities
Tsartali, et al. [37]	2008	Cross-sectional study of Greek adolescents from the island of Chios	200	15-17	KIDMED	BMI	Logistic regression model	No significant association between MD adherence and BMI (p=0.506)	-
Garcia-Marcos, et	2007	Cross-	20106	6-7	MDS	BMI, IOTF	U-test	Significantly	

Reference	Year	Study	N	Age	Exposure	Outcome	Analysis	Result	Adjusted for
al.[38]		sectional study of Spanish schoolchildren				definition of obesity		different MDS between obese and non-obese ($z=4.13$, $p<0.001$ for girls)	

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