

Associations between the adherence to the Mediterranean diet and cardiorespiratory fitness with total and central obesity in preschool children; the PREFIT project

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KEYWORDS: preschool children, obesity, adherence to the Mediterranean diet, cardiorespiratory fitness, dietary habits.

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40 **ABBREVIATIONS**

CRF: Cardiorespiratory fitness

CVD: cardiovascular disease

KIDMED: Mediterranean Diet Quality Index for children and teenagers

MDP: Mediterranean dietary pattern

45 PREFIT: Assessing FITness in PREschoolers

WOF: World Obesity Federation

ABSTRACT

PURPOSE: Early recognition of risk factors associated with overweight/obesity are important steps towards preventing long term health consequences. The aim of the current study was to examine the associations of the adherence to the Mediterranean dietary pattern (MDP) and cardiorespiratory fitness (CRF) with adiposity in preschool children from North of Spain.

METHODS: The adherence to the MDP (KIDMED), CRF (20-m shuttle-run-test), total (BMI) and central (waist circumference) adiposity and socio-demographic factors were assessed in 619 children (48.6% girls) who were on average 4.7 years old.

RESULTS: Higher MDP index ($P<0.05$) and CRF levels ($P<0.01$) were significantly related to lower waist circumference. CRF was inversely associated with BMI ($P\leq 0.001$), yet no significant association was observed between MDP and BMI. Children non-having high CRF levels and high to the MDP (i.e., non-upper sex-specific tertile of CRF or MDP, respectively) had the highest waist circumference.

CONCLUSIONS: Our findings support that higher adherence to a MDP and higher CRF are associated with lower waist circumference in preschool children, pointing them as relevant modifiable factors to be targeted by educational strategies aiming to prevent central obesity and later obesity-related comorbidities.

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KEY WORDS: preschool children, obesity, adherence to the Mediterranean diet, cardiorespiratory fitness, dietary habits

INTRODUCTION

70 The manifestation of obesity in children is occurring at progressively younger ages worldwide [1]. This is of great concern from a public health perspective due to the tracking of childhood obesity into the adulthood. Moreover, there is strong relationship between the number of years lived with overall [2] or abdominal obesity [3] and the risk of cardiovascular disease (CVD) mortality and all-cause and mortality. Hence, the results of the Framingham Cohort Study
75 showed that the risk of CVD mortality increased 7% for every 2 years additional lived with obesity [2].

The rise in the prevalence of overweight/obesity in preschool children has been particularly dramatic in the last three decades [4]. Likewise, the increase of early childhood overweight/obesity worldwide was 21% from 1990 to 2000, and 31% from 2000 to 2010[5]. In
80 2010, the Early Childhood Longitudinal Study reported that almost 15% of kindergarten-age children were overweight and 12.4% obese in the United States [6]. The latest systematic review in Europe reported prevalence rates of overweight or obesity between 8.4% and 31% in 2-5 years children, and observed the highest rates in countries in the Mediterranean region [7]. Spain, together with Malta and Sicily is leading the ranking of overweight and obesity rates in
85 children aged 5-10 years [8].

Childhood is a crucial period in life in which individuals adopt lifestyle patterns that are likely to track into their adulthood. Healthy dietary habits and adequate physical activity levels during early life reduce the risk of major chronic diseases later in life [9]. Physical activity level, particularly moderate to vigorous physical activity, is strongly associated with cardiorespiratory
90 fitness (CRF), a powerful health marker. However, in the last decades, the consumption of unhealthy diets has become average dietary patterns [10] and physical activity levels, and consequently CRF, have decreased among children [11]. Low level of CRF is engaged in the etiology and occurrence of many chronic non-communicable diseases, such as cardiovascular diseases, diabetes or obesity. For instance, higher total and central adiposity have been
95 associated with low levels of CRF in children and adolescents [12]. This is potentially of great interest and concern because CRF is an important marker of health already in childhood.

The Mediterranean dietary pattern (MDP), based on the typical dietary habits followed by people from countries around the Mediterranean Sea, has been extensively studied due to its health benefits [13]. High adherence to the MDP may reduce major chronic disease morbidity and mortality [13], and with lower adiposity and overweight prevalence in youths [14,15], while other found no significant associations between the adherence to the MDP and obesity prevalence[16]. Interestingly, epidemiological evidence suggests that dietary patterns in the South of Europe are changing especially among youths, and that children living in countries in the South of Europe can even have lower adherence to the MDP than their peers living in the North of Europe [14].

The majority of the studies examining the influence of CRF level and the adherence to the MDP in overweight/obesity risk have been conducted in school children or adolescents. There are no previous epidemiologic studies examining the influence of both the adherence to the MDP and CRF levels on adiposity measures in preschool children. Early recognition of risk factors associated with overweight and obesity and monitoring overweight/obesity rates are important steps towards preventing long term health consequences. Therefore, the aims of the current study were: 1) To examine the adherence to the MDP in preschool children; and 2) To examine the associations of the adherence to the MDP and CRF with body mass index (BMI) and waist circumference in a sample of preschool children from the North of Spain.

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METHODS

Design and participants

The PREFIT study (Assessing FITness in PREschoolers) is a multicentre cross-sectional study performed in 11 towns/cities of Spain (Almería, Cádiz, Castellón, Cuenca, Granada, Las Palmas de Gran Canaria, Madrid, Palma de Mallorca, Zaragoza, and Vitoria-Gasteiz) that aimed to evaluate nutritional status and fitness in preschool children (Supplemental Figure 1). The present study focused on the sample from Vitoria-Gasteiz (North of Spain), the only center in which the adherence to the MDP was studied, and it is not representative of the whole Spanish population of preschoolers. This study therefore comprised 619 children (8% of the children aged 3-5 years living in the city, 48.6% girls) aged 4.7 ± 0.8 years recruited in six schools, three public (N=328) and three private (N=291). Participation rate was lower in children aged 3 years (55.4%), than in 4 (77.8%) or 5 (77.4%) years old children (Supplemental Figure 2). The reason for non-participation was not registered. Data collection took place from March to November 2015.

The protocol of the study was approved by the Human Research Ethics Committee of the University of the Basque Country. Detailed information about the aims of the study and tests, as well as questionnaires and informed consent sheets, were delivered to the families by the schools participating in the study. All the parents sent the informed consent for their children's participation in the study to the schools.

Anthropometric measurements

Waist circumference, body mass (kg) and height were measured following standard protocols and body mass index (BMI) was calculated. BMI was categorized into underweight, normalweight, overweight and obesity according to the World Obesity Federation (WOF) criteria[8]. Sex-specific z-score of BMI and waist circumference were calculated as follows: $(\text{data} - \text{mean of the sample}) / \text{standard deviation of the sample}$.

Adherence to the Mediterranean diet

The adherence to the MDP was assessed by using the KIDMED questionnaire (Mediterranean Diet Quality Index for children and teenagers)[17]. This test has been previously validated and

145 it is widely used in childhood [17]. Since the participants were too young to fill the
questionnaire, their parents or legal guardians were asked to answer the questions on their
children dietary habits. The test is composed of 16 nutritional items, 10 questions about the
consumption of the different food groups and six more about healthy dietary habits not directly
associated to the MDP [14]. For the purpose of the current study only those items related to the
150 MDP were considered, while questions about breakfast habit, eating in fast food restaurants or
taking sweets were not included in the main analyses (**Supplemental Table 1**). A value of 1
was given to the questions which have a positive connotation in accordance to the MDP. The
total MDP score (MDP index) was computed by adding up all the values obtained in the 10
items. Thereby, the MDP index test ranged from 0 to 10 points. The MDP score was also
155 expressed as percentage of adherence. Optimal adherence to the MDP when the MDP index was
 ≥ 8 or the percentage of adherence to the MDP was $\geq 80\%$)

Cardiorespiratory fitness

The level of CRF was estimated using the PREFIT 20-m shuttle run test as described elsewhere
[18]. This test was adapted from the original protocol mainly reducing the initial speed from 8.5
160 km/h to 6.5 km/h. Briefly, children started the test at 6.5 km/h and the speed increased 0.5 km/h
per minute. The test finished when the children failed to reach the end line concurrent with the
audio signal on two consecutive occasions or when the child stop because of exhaustion.

Potential confounding factors

The following socio-demographic variables have been previously shown to be associated with
165 obesity, dietary habits and/or CRF in childhood and were considered as potential confounders:
maternal and paternal educational level ([1] non-university: lower education, lower secondary
school and higher education and [2] university degree), and ethnicity that was defined as the
country of origin of the mother and was categorized for statistical analyses as: 1=Spanish and
2=non-Spanish.

170 *Statistical analyses*

Differences in anthropometric, socio-demographic, dietary and CRF characteristics between
boys and girls were examined using t-Student tests for independent samples (continuous

variables) or Chi-square tests (categorical variables). The distribution of all the continuous variables was tested for normality.

175 To investigate associations between the MDP index and CRF levels with BMI, BMI z-score, waist and waist z-score linear regression analyses were conducted. Two regression models were created: (1) model adjusted with age and sex (Model 1), and (2) model adjusted with age, sex and socio-demographic variables (Model 2). Analyses examining the associations with waist circumference and waist circumference z-score were additionally controlled for
180 height, while CRF was further controlled for body mass. We also examined interactions by sex including interaction terms into the models; as there were no significant interactions ($P > 0.05$), the results for boys and girls are presented together.

Differences in adiposity between having high adherence to the MDP (within the upper sex-specific tertile of the MDP index) and non-having high adherence to the MDP (non-upper
185 sex-specific tertile of the MDP index) were explored by ANCOVA adjusting with age, sex and socio-demographic variables (basic covariates). Similarly, having high CRF levels (within the upper sex-specific tertile of CRF) *vs.* Non-having high CRF levels (non-upper sex-specific tertile of CRF) were also analysed by ANCOVA controlling with basic covariates. The existence of an interaction effect between the MDP index and CRF on anthropometric variables
190 was also tested by ANCOVA adjusting with the same covariates. Thereafter, the combined influence of having or non-having high adherence to the MDP and having or non-having high CRF on anthropometric variables was examined by ANCOVA adjusting with age, sex and socio-demographic variables. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 21.0 for WINDOWS; SPSS Inc, Chicago), and the level of
195 significance was set at $\alpha = 0.05$.

RESULTS

Socio-demographic and anthropometric characteristics, as well as CRF, separated for boys and girls are shown in **Table 1**. It was observed that 4.7% (4.1% in boys and 5.3% in girls) of preschool children had insufficient body weight, 16% were overweight (15.2% in boys and 16.9% in girls), and 4.9% were obese (3.5% in boys and 6.3% in girls). Boys were taller ($P<0.05$), had lower waist circumference ($P<0.05$) and higher CRF level ($P<0.001$) than girls.

Dietary habits of study participants are shown in **Supplemental Table 1**. The mean adherence to the MDP was higher in girls than in boys ($p<0.001$, Table 1). It was observed that less than 25% of participants had at least 80% of adherence to the MDP (24.3%, 32.3% of girls and 17.2% of boys) and that 35% of preschool children did not exceed 50% of adherence to the MDP (29.7% of girls and 39.7% of boys, **Supplemental Figure 2**). Only 2.4% of preschoolers showed 100% of adherence to the MDP (3.9% of girls and 1.0% of boys).

210 *Adherence to the Mediterranean diet, cardiorespiratory fitness and adiposity*

A higher adherence to the MDP was significantly related to lower waist and waist z-score regardless of sex, age and height (Model 1, **Table 2**) and age, sex, height, and sociodemographic factors (Model 2, Table 2). A higher CRF level was significantly associated with lower BMI, BMI z-score, waist and waist z-score regardless of sex and age (Model 1, **Table 2**) and age, sex, and sociodemographic factors (Model 2, Table 2). Likewise, waist and waist z-score were lower in preschoolers within the upper sex-specific tertile of the adherence to the MDP ($P<0.05$, Figure 2) and in those children within the upper sex-specific tertile of CRF than in their peers with lower CRF levels ($P<0.01$, **Figure 1**).

There were not found significant interaction effects between the adherence to the MDP and CRF level on BMI and BMI z-score ($P>0.05$). However, there were observed significant interaction effects between the adherence to the MDP and CRF level on waist ($P=0.013$) and waist z-score ($P=0.008$). Therefore, it was examined the combined influence of having or non-having a high adherence to the MDP (upper or non-upper sex-specific MDP index) and having or non-having high CRF levels (upper or non-upper sex-specific tertile of CRF) in waist and

225 waist z-score (**Figure 2**). The results showed that both waist and waist z-score were
significantly higher in those children non-having both high CRF levels and high MDP index
than in those non-having high CRF levels, but with high MDP index ($P=0.034$ and $P=0.040$, for
waist and waist z-score, respectively) independently of age, sex, height squared, and
sociodemographic factors. Moreover, children non-having high CRF levels and non-having high
230 MDP index had higher waist and waist z-score than those having high CRF levels and high
MDP index ($P=0.020$ and $P=0.003$, for waist and waist z-score, respectively), or non-high MDP
index ($P=0.016$ and $P=0.008$, for waist and waist z-score, respectively), regardless of covariates.
However, there were no significant differences in waist and waist z-score between children
having high CRF and high MDP index and those having high CRF but non-high MDP index.

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DISCUSSION

In the current study, BMI and waist circumference, the adherence to the MDP and CRF were examined in more than six hundred Spanish preschool children. The study aimed to explore the association of the adherence to the MDP and CRF levels on adiposity estimates. The main findings were (1) the adherence to the MDP was low to moderate; (2) higher adherence to the MDP was associated with lower waist circumference, while higher levels of CRF were associated with both BMI and waist circumference; (3) those children with low adherence to the MDP and low CRF had higher waist and waist z-score, regardless of age, sex and sociodemographic factors. To the best of our knowledge, our study is the first examining the combined associations of the adherence to the MDP and CRF with total and central adiposity estimates in preschool children.

In the current study, 16.0% of preschool met the *criterium* for overweight and 4.9% for obesity. These results reinforce findings from other studies demonstrating that Spain, together with other Mediterranean countries, showed the highest prevalence of overweight and obesity in early childhood compared with other European countries, particularly Scandinavian countries[19,20]. Likewise, overweight/obesity rates in our study sample were similar to those recently reported in a study of 128 preschoolers from the South of Spain (22% classified according to the WOF criteria)[20] and also to the percentage of overweight children aged 2 to 10 years identified as overweight or obese (21.2%) in the Spanish children participating in the IDEFICS study [21].

Findings of the current study support previous evidence for low to moderate adherence to the MDP among children [22]. Likewise, the average adherence of the preschoolers to the MDP was 62.7% and only 24.3% of children showed optimal adherence ($\geq 80\%$). The low proportion of preschoolers with optimal adherence to the MDP observed in the present study is of concern and confirms that children living in Mediterranean countries are abandoning the traditional MDP [22]. Optimal adherence to the MDP in the current report was lower than in Spanish preschool children participating in the IDEFICS study (32.3%), whose data were obtained eight/nine years earlier in Zaragoza, one city located also in the North of Spain close to

Vitoria-Gasteiz [14]. Although it cannot be interpreted as a secular trend of worsening dietary habits among preschool children, it seems that at least in the North of Spain the adherence to the MDP is decreasing in early childhood, in agreement with previous findings. Results from older children are even more alarming. In Italian 8-9 years old children, only 5.0% had optimal adherence to the MDP [23]. In older children (from 10 to 12 years old), several studies conducted in large samples of Greek [16], Cyprus [24] and Balearic Islands [25] reported optimal adherence to the MDP ranging from 4.3% to 7.1%. To note is that direct comparison among studies are difficult because of differences in the age of children and methods used to assess the adherence to the MDP. Indeed, three of the above mentioned studies assessed the MDP with the KIDMED questionnaire [16,23,24] and two with food frequency questionnaires [14,25].

We observed that lower adherence to the MDP was associated with higher waist circumference, which in turn is strongly related to cardiometabolic risk. The specific relationships between dietary factors and/or diet quality and abdominal and truncal fat mass, as well as with visceral and hepatic fat regardless of total body fat, have been previously reported in several studies [26-28]. The influence of the adherence to the MDP on waist circumference in older pediatric populations was previously reported in cross-sectional [29] and longitudinal studies [14], though no significant associations have also been shown [16,30]. The observed association of MDP with waist circumference in preschoolers may help to explain the previously reported relationship between the adherence to the MDP and metabolic syndrome in older children and adolescents [31], as well as with the preventive role of the MDP against cardiovascular disease later in life [13]. Given the influence of the MDP on the prevention of chronic diseases, and with the reduction in total mortality and improvement of longevity [13], health policies should focus on promoting this traditional dietary pattern from early childhood.

We observed that a worse performance in the 20m shuttle run test (i.e., CRF) was associated with higher BMI and waist circumference in preschoolers. Our results agree with previous studies conducted in school children and adolescents [11]. Furthermore, these findings concur with the few reports that have examined the association of CRF with central and overall adiposity in preschoolers [32,33]. Of note that CRF is a physical condition, while dietary pattern is a behavior. However, despite the strong genetic component of the CRF, regular physical activity (a lifestyle behavior) is one of its main determinants [34]. Therefore, our results

295 reinforce the importance of promoting physical activity already in preschool children. Excess
body fat gain is determined by a positive energy balance; thus, the increase of physical activity
level may have benefits improving CRF, but also increasing energy expenditure.

The most novel observation of the current study is the combined influence of the
adherence to the MDP and CRF on waist circumference in preschoolers. The results showed
300 that the highest levels of waist circumference were found in those children who were unfit and
had an unhealthy diet. It can be also observed that children non-having high CRF levels had
higher waist and waist z-score than those with high CRF, regardless of their adherence to the
MDP. However, it should be also noted that children non-having high CRF and with low
adherence to the MDP had higher waist circumference than those non-having high CRF but
305 with a high adherence to the MDP. Actually it means that those children with non-high CRF
levels and non-having a high adherence to the MDP are at a higher risk of central obesity and
cardiometabolic risk later in life. These findings expand the current knowledge since, as far as
we are aware, this is the first study reporting both the separate and combined influence of the
adherence to the MDP and CRF on adiposity estimates in preschool children.

310 The current study has several limitations. Findings from our study should be taken with
caution due to its cross-sectional design. Therefore, longitudinal studies are needed to confirm
the long term influence of the adherence to the MDP and CRF on obesity and adiposity gain.
Collection of dietary data for more than two days or the use of a food frequency questionnaire
would have been desirable to compensate for day-to-day variability. However, the KIDMED
315 has been previously validated to use in children [17] and chosen as the dietary assessment
method to estimate the adherence to the MDP in many previous studies [16,23,24]. Specifically
in preschool children, it has the advantage of its short administration time and high response
rate. The sample is not representative of the population of the North of Spain; however, it is a
relatively large sample size that covers both private and public schools that should be
320 acknowledged.

CONCLUSIONS

The current study observed poor adherence to the MDP among children from 3 to 5 years together with a high prevalence of overweight and obesity ($\approx 21\%$). Our results demonstrate that only CRF was negatively related to total adiposity; however, both non-high adherence to the MDP and non-high CRF levels, are associated with higher waist circumference already in preschoolers. Likewise, children non-having high CRF levels and with low adherence to the MDP had the highest levels of waist circumference. Overall, these findings suggest that educational strategies aimed to increase CRF and promote healthy dietary habits are necessary from the early childhood to prevent children from suffering excess total and abdominal adiposity and later cardiovascular disease.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist

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FIGURE LEGENDS

480 **Figure 1.** Influence of having high CRF (upper sex specific tertile of cardiorespiratory fitness) or non-high CRF (non-upper sex specific tertile of cardiorespiratory fitness) and having high MDP index (upper tertile of sex specific MDP index) or non-having high MDP index (non-upper sex-specific tertile of the MDP index) on waist circumference and waist circumference z-score in preschool children. Analyses were adjusted with age, sex, maternal and paternal
485 education level, ethnicity and height squared (covariates). MDP index: adherence to the Mediterranean dietary pattern. Values are adjusted means \pm 95% CI.

Figure 2. Combined influence of having high CRF (upper sex-specific tertile of cardiorespiratory fitness) or non-having high CRF (non-upper sex-specific tertile of cardiorespiratory fitness), and having high MDP index (upper sex-specific tertile of the MDP
490 index) or non-having high MDP index (non-upper of sex-specific tertile of the MDP index) on waist circumference and waist circumference z-score in preschool children. Analyses were adjusted with age, sex, maternal and paternal education level, ethnicity and height squared (covariates). MDP index: adherence to the Mediterranean dietary pattern. Values are adjusted means \pm 95% CI.

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