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RESEARCH

Mediterranean diet, physical activity, and family characteristics associated with cognitive performance in Italian primary school children: analysis of the I-MOVE project

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Abstract

Working memory (WM) is a multicomponent system that supports cognitive functioning. It has been linked to a wide variety of outcomes including academic success and general well-being. The present study examined the relations between adherence to the Mediterranean diet (MD) and WM among Italian children, adjusting for important parent characteristics and children's lifestyle habits. Data for this study was obtained from 106 children attending primary school in Imola (Italy) who were part of the I-MOVE study emphasizing school-based physical activity. Children's adherence to the MD was calculated using the KIDMED index (KI) based on the ZOOM-8 questionnaire. Physical activity (PA) levels were assessed using an actigraph accelerometer and WM using the backward digit span test. Univariate regression was used to select significant child-level and family measures associated with WM, which were then tested in a single multivariate regression model. Older age is positively associated with higher WM ($\beta = 0.36$; 95% CI 0.25, 0.47). Dietary adherence (KI) ($\beta = 0.07$; 95% CI 0.01, 0.14) and engagement in organized PA outside school hours ($\beta = 0.58$; 95% CI 0.09, 1.10) are positively related to WM. Among the family measures, father's education was positively associated with WM for high school education and for university vs. middle school or lower, respectively.

Conclusion: Adherence to the MD was associated with better WM capacity in primary school children. These findings can be used to guide policymakers in designing health promotion programs and instituting policies emphasizing healthy nutrition to improve physical health and boost cognitive functioning.

What is Known:
- *The development of working memory involves the entire childhood with a rapid spurt between 2 and 8 years of age.*
- *Working memory plays a critical role in children's learning and academic performance and underlies higher-order cognitive abilities.*

What is New:
- *Adherence to the Mediterranean Diet was associated with higher working memory capacity in primary school children.*
- *Health promotion interventions based on PA and sound nutrition involving children benefit not only physical and mental health, but also cognitive health.*

Keywords Working memory · Mediterranean diet · Physical activity · Lifestyle · Parental education

Introduction

The past decade has witnessed a growing body of literature suggesting that numerous individual, behavioral, and socioeconomic factors are related both to general intelligence and cognitive functioning in adults and children [1, 2]. The same literature also suggests that healthy lifestyle factors are positively associated with the development of executive "functions" (i.e., working memory [WM], flexible thinking,

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and self-control) in schoolchildren [3–5]. This is important because executive functions like WM play a fundamental role in children's learning and academic performance and lay the foundation for higher-order cognitive skills and success later in life [6].

Across the lifespan, WM, in particular, is considered a core element of human cognition. It entails the ability to retain and hold a small amount of information in a readily accessible form and maintain the required information "in mind" while engaged in mental processing [7, 8]. Working memory has been established as one of the best predictors of general intelligence [9] and is related to information processing, comprehension, problem-solving, learning, and knowledge acquisition [10]. Given its vital role, WM has also been linked to a wide variety of important life outcomes including academic success [11–16], income [17] health, and well-being [18, 19].

Because of its fundamental role in mental processing, WM is quite prominent for children in education and learning [20]. Specifically, WM may play a role in academic achievement [21], cognitively demanding tasks (i.e., reading comprehension, language abilities, mathematics, reasoning, and also overall academic performance [22, 23]. Developmentally, WM starts to develop in the 1st year of life, undergoes a rapid spurt between 2 and 8 years of age [6, 24], and continues to develop through adolescence.

Given the pivotal role played by cognitive performance and executive function in a person's life, from a public health perspective, it is essential to identify factors that may influence the core elements that sustain cognitive development [6]. Recent studies have identified both modifiable and non-modifiable factors including gender, age, personality, mental illness, medical conditions, dietary habits, stress, sleep, exercise, and brain stimulation [25]. Among these putative risk and protective factors, dietary habits are amenable to change through intervention. Indeed, there is evidence that adherence to the Mediterranean Diet (MD) can influence children's academic performance [26, 27] and cognitive functioning [28, 29]. As important as this evidence is to support nutritional interventions, these studies are limited to single meals (e.g., breakfast [30, 31]), specific micronutrients (e.g., omega 3 and omega 6 [32, 33]), or specific diets. The Mediterranean Diet represents more of an "approach" to achieving sound nutrition entailing the "organization" and daily intake of multiple food groups (i.e., vegetables, fruits, whole grains, beans, olive oil, to name a few). In light of this, more research is needed that examines the impact of healthy food groups on cognitive functioning in children to inform rationally sound intervention strategies [34, 35]. To the best of our knowledge, the current study is the first to investigate relations between adherence to the MD and WM in a sample of Italian primary school children.

Factors outside of diet and nutrition can also influence both a child's adherence to a specific eating plan and their cognitive functioning. For instance, a child may eat certain food groups because their parents offer these foods on the table, or encourage eating healthy as part of their daily routine (this can include snacks at school). Parent's education may also play a role in determining adherence to sound nutrition as well as the child's overall cognitive abilities. This relation arises because more educated parents may have greater knowledge about sound nutritional practices and make them part of the child's daily repertoire. Moreover, more highly educated parents can stimulate their child's memory by providing greater resources in the home. This can include toys when they are young, reading to them during early childhood, and continued mental stimulation throughout development. Ultimately, food availability plays a role in a child's weight and sound nutrition plays a role in growth, both of which factor into a child's BMI, which in turn can influence the amount of physical activity they engage. Taken together, all of these factors may interrelate and either mask the influence of diet by itself or independently contribute to cognitive and learning outcomes. In summary, this study aims to relate dietary adherence (MD) to WM. To address the relations between dietary adherence and WM, we examined data obtained from a larger health promotion trial (I-MOVE) being conducted with Italian school children to stimulate PA as part of classroom activities.

Methods

Study design and participants

This cross-sectional study was conducted among a sample of children and their parents enrolled in the "I-MOVE" project [36]. The children were attending a primary school in Imola (Bologna), located in the Emilia Romagna Region in the North of Italy. The Bioethics Committee of the University of Bologna approved the "I-MOVE" on March 18, 2019 (0054382). The study was conducted following human subject protection guidelines in the Declaration of Helsinki. A researcher explained the study to a group of parents (and teachers) during an afternoon following a school day. Parents could ask questions and express any concerns regarding the involvement of their children in the study. At that point, the researcher provided parents with informed consent forms for their children to participate in the study. Of four schools approached in Imola one agreed to participate in the I-MOVE project [36]. From this school, five classes out of 15 agreed to be involved in the project. All of the children from the 1st to the 5th grades with no health problems or physical disability, which might limit PA performance, and whose parents provided consent were available to participate in the study. The study was designed following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines [37].

Instruments

Data were collected from October 2019 to December 2019 using the following instruments: ZOOM-8 questionnaire [38], actigraph accelerometers [39], and WM assessment [40].

The ZOOM-8 questionnaire is a self-report instrument that assesses dietary habits and food consumption, with a small section set aside to collect information on the parent's and child's lifestyle (retrospectively up to 1 year), the latter including PA levels and sedentary behaviors [38]. The instrument contains two different sections: The first section involves a general questionnaire that assesses personal information (i.e., parent educational levels), and lifestyle (i.e., the child's weight, height, amount of PA, time spent using computers or watching TV, sleeping hours, family composition). The second section contains a semi-quantitative food frequency questionnaire (FFQ), the latter following the methodology described and validated by Willett [41]. The FFQ consists of 53 commonly used food items categorized into 11 food groups [42]. Frequency response categories for all food portions ranged from the number of times per day, per week, per month, and per year to never. Manufactured products are reported as the number (or fraction) of units consumed. Before completing the questionnaire, standardized pictures were shown to both children and parents to clarify the definition of what is meant by "portion."

Anthropometric status was calculated according to the International Obesity Task Force (IOTF) cut-off values [43, 44]. The IOTF provides weight categories based on BMI (meters^2/kg) stratified by age and sex. We used this algorithm to derive two categories including underweight/normal weight and overweight/obese. Caregivers filled out the questionnaire based on their own PA and also reported PA levels for their spouse, necessitating that we create a dichotomous variable (yes = at least one of the parents does PA once a week, no = none of the parents engage in PA once a week). Responses to questionnaires were anonymous, evaluated for accuracy and completeness by researchers, and subsequently analyzed. In order to reduce bias, parents were instructed by members of the research team on how to independently fill out the instrument at home.

Physical activity levels

Daily PA levels and sedentary behavior of the children were monitored over a 7-day period (5 weekdays and 2 weekend days) using actigraph accelerometers model GT3X (ActiGraph LCC: Pensacola, FL, USA). We examined the accelerometer data using ActiLife 6.13.3 software (ActiGraph LCC: Pensacola, FL, USA). The epoch length was set to 10 s to allow a detailed estimate of PA intensity [39]. Children wore the accelerometers around the waist with an elastic belt and were instructed to remove them only when bathing, swimming, or showering [45]. Minutes spent in PA (converted to light, moderate, and vigorous) per day were calculated using the Evenson cut-points [46].

Working memory

Working memory was assessed using the backward digit span, a subtest of the Wechsler Intelligence Scale for Children (WISC-IV) [40]. The highly reliable task involves verbally presenting a digit series and requires children to repeat the series in reverse order. The score was calculated as the highest number of correct digits remembered.

Dietary assessment

We evaluated children's lifestyles by investigating several different factors. We calculated adherence to the MD using the modified Diet Quality Index for Children and Adolescents (KIDMED) score [47, 48] by extracting data from the ZOOM-8 questionnaire [38]. Essentially, the various pieces of information in the Zoom-8 regarding nutritional food consumption is categorized (i.e., vegetables, pasta, fish, cheese, milk products, legumes, grains, fruit, dried fruit, nuts, snack, candies, and olive oils) and assigned a numerical equivalent. This is added to information about skipping meals (breakfast), and frequency of consumption, both of which are made numerical (yes/no). We then classified children into three distinct categories based on their KIDMED index [49]: (1) > 8–12, high; (2) 4–7, medium, and (3) 0–3, poor. We selected the KIDMED Index to assess adherence to the MD because it is easy to interpret and provides the end user with a comprehensive (numerical) overview of an individual's dietary intake (and adherence to the nutritional plan).

Statistical analysis

The present manuscript is a cross-sectional snapshot of sample behavior taken within the larger I-MOVE project. The sample size calculation has been performed for the primary analysis. A post-hoc power analysis was conducted to determine if the sample size was adequate to estimate the multiple regression model presented. Continuous variables are reported using mean and standard deviation (± SD) and categorical variables using absolute and relative frequencies. The normal distribution of the selected variables was assessed with the Shapiro–Wilk test and their distribution was examined using density graphs. Records with missing data on the dependent variables were excluded from the analysis. Missing data on independent variables were treated as missing at random.

Univariate analysis (Student *t*-test, analysis of variance, and linear regression) was performed modeling WM score as the dependent measure. A regression model with Akaike criterion selection was then employed to identify the independent factors associated with the continuous dependent measure. The original set of variables was chosen based on the univariate analysis results.

The significance level was set as $p < 0.05$. All analyses were carried out using R version 4.1.3 (R Project for Statistical Computing) [50].

Results

Main results

The I-MOVE study involved a sample of 153 children. A total of 47 records were discarded because the WM outcome measure was not reported, resulting in 106 children available for the current analysis. The sample consisted of 53 (50%) girls and 53 (50%) boys, ages 6 to 10 years (mean 7.92 ± 1.40). More than half of the sample had normal weight ($n = 66$; 63%) and according to Cole cut-off values by sex and age, the remaining children were rated as overweight/obesity ($n = 39$; 37%). The average WM score was 3.12 (± 1.04) and the average KIDMED score was 4.44 (± 2.28). A majority of the children regularly participated in organized sports outside school hours ($n = 94$; 89%). Table 1 summarizes the sample characteristics and weekly habits of the children.

Univariate analysis showed a significant positive relationship between a child's age ($p < 0.001$), KIDMED Index category ($p = 0.033$), and WM score (Fig. 1). Moreover, children who engaged in organized physical activities outside of school hours had a significantly higher ($p = 0.032$) WM score (3.22 ± 0.97) as compared to children who were not engaged (2.33 ± 1.23). Similar patterns were uncovered for children with normal weight (3.30 ± 1.04) compared to children rated as overweight/obesity (2.85 ± 0.99; $p = 0.027$), and children whose fathers had a higher educational level ($p = 0.001$). The complete set of univariate analyses for both categorical and continuous measures can be found in the Supplemental material (Tables S.1 - S.2).

Table 2 contains the results of the final multivariate linear regression model (and should be read in conjunction with Supplemental Tables S.1 and S.2). IOTF class and KIDMED Index were colinear, hence we decided to include only the Kidmend Index in the final model. As expected, older age is positively associated with higher WM scores ($\beta = 0.36$; 95% CI 0.25, 0.47). Similarly, a child's higher KIDMED Index score ($\beta = 0.07$; 95% CI 0.01, 0.14) and their engagement in organized sports activities outside of school hours ($\beta = 0.58$; 95% CI 0.09, 1.10) were both positively related to the WM score. Among the parent characteristics, only father's educational level (reference class, "middle school or lower") was positively associated with a higher WM score ("high school" $\beta = 0.57$; 95% CI 0.14, 1.0, and "university degree or higher" $\beta = 0.79$; 95% CI 0.30, 1.3). The final regression model was adequately powered > 0.95.

Discussion

A child's diet can influence their well-being in many ways. One way is that diet can make them feel better and be positively associated with cognitive functioning. In this study, we investigated the lifestyle habits of primary school children (diet, PA, and sedentary behavior), parent influences, and their association with WM performance. Working memory undergirds cognitive functioning because it represents memory capacity and is instrumental in a wide range of mental functions including information processing, comprehension, problem-solving, and knowledge acquisition. These cognitive functions are fundamental to success in later life and form the substrate on which sound decision-making, well-being, and healthy living are built. Importantly, we found that age, adherence to the MD, engagement in organized sports, and father's educational level are related to WM in children. Notably, we used the KIDMED Index as the index of choice for investigating adherence to the MD because it is an easy-to-interpret index that gives the reader a comprehensive overview of an individual dietary intake, rather than focusing on a single food or food group. Thus, we took a more expansive view of adherence to sound nutritional practices using the KIDMED index.

The current findings reinforce what has been reported in the literature suggesting that dietary adherence and engagement in organized sports can positively influence children's cognitive performance [28]. Moreover, the findings also suggest that family-based factors such as the father's education also can play a significant role in determining a child's WM. Other authors have previously examined these relations but have limited their studies to specific meals, food groups, or specific subpopulations [30, 31]. To our knowledge, this is the first article addressing the impact of social and behavioral determinants on WM in a sample of parents and their children. One important question is whether the sample is representative of the larger Italian population from which it is drawn. To accomplish this, we used the OKkio alla

Table 1 Population characteristics

Study population	$N = 106$
Gender	
Females	53 (50%)
Males	53 (50%)
Age, years (mean ± SD)	7.9 ± 1.4
IOTF category	
Normal weight/underweight	72 (68%)
Overweight/obese	33 (32%)
Missing	1
Night sleep, hours (mean ± SD)	9.1 ± 0.8
Working memory (mean ± SD)	3.1 ± 1.0
KIDMED index	4.4 ± 2.3
KIDMED categories	
Low	35 (33%)
Average	59 (56%)
Optimal	12 (11%)
Sport participation outside school hours	
No	12 (11%)
Yes	94 (89%)
Average daily sedentary activity, minutes (mean ± SD)	943.0 ± 55.4
Average daily light activity, minutes (mean ± SD)	244.5 ± 41.7
Average daily moderate activity, minutes (mean ± SD)	31.3 ± 10.9
Average daily vigorous activity, minutes (mean ± SD)	16.8 ± 8.3
Average daily number of steps (mean ± SD)	8104.9 ± 2234.6
More than 60 min of daily moderate-vigorous physical activity	
No	65 (61%)
Yes	41 (39%)
Mother age, years (mean ± SD)	39.8 ± 5.3
Missing	1
Father age, years (mean ± SD)	42.9 ± 5.5
Missing	1
Mother BMI category	
1 Underweight/normal weight	78 (79%)
2 Overweight/Obese	21 (21%)
Missing	7
Father BMI category	
1 Underweight/normal weight	41 (42%)
2 Overweight/obese	56 (58%)
Missing	7
Mother educational level	
1 Middle school or lower	13 (12%)
2 High School	50 (48%)
3 University degree or higher	42 (40%)
Missing	1
Father educational level	
1 Middle school or lower	19 (18%)
2 High School	69 (56%)
3 University degree or higher	27 (26%)
Missing	1
At least one parents exercise regularly	
No	57 (54%)
Yes	49 (46%)

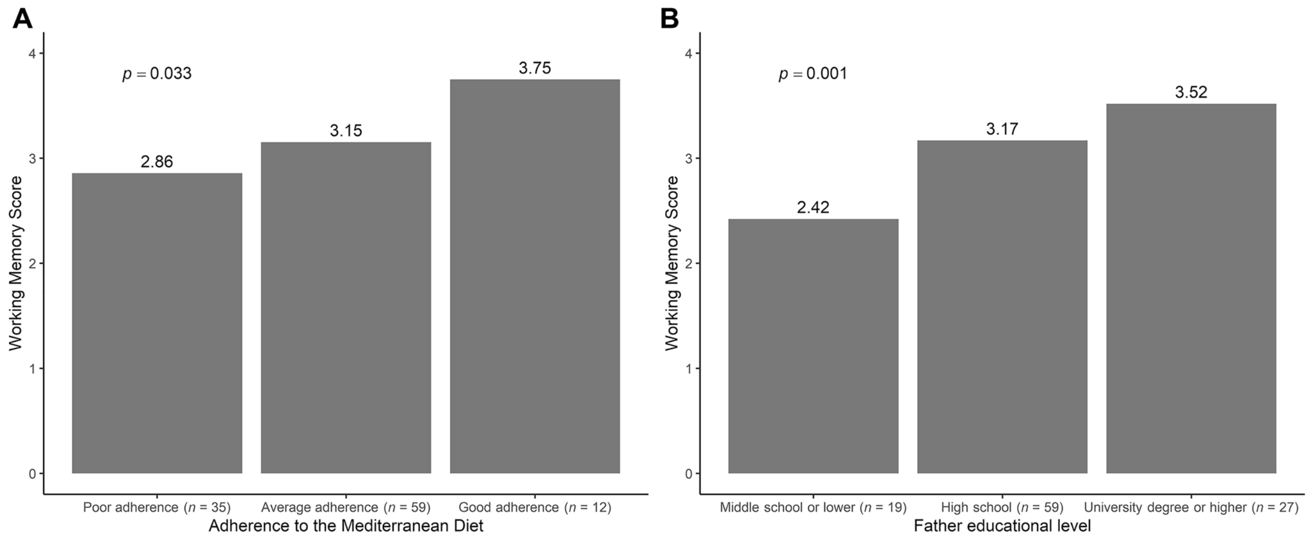

Fig. 1 Working memory score and adherence to the Mediterranean diet (**A**), and father educational level (**B**)

SALUTE, an Italian epidemiological surveillance system funded by the Ministry of Health that gathers data on overweight and obesity status among primary school children [51]. As a basis of comparison, in our sample, 32% of the children presented as overweight/obese. This is slightly higher compared to the reported prevalence of obesity in the Emilia-Romagna primary school population (29.8%) [51]. The KIDMED index for our sample was consistent with other Italian primary school children's dietary assessments, with only 33% of individuals reporting poor adherence to the Mediterranean Diet [38, 52, 53]. When we examine PA levels measured by actigraph accelerometers, 39% of our sample met the recommended levels of 60 min of MVPA every day. This finding is quite similar to data from a recent European study using accelerometers as an objective measure of PA. The authors found that only up to 29% of children are categorized as sufficiently PA [54]. In Italy, OKkio alla SALUTE [51] reported only 20.3% of children do not reach the PA recommendations on the day before the survey was fielded in their regions. However, the data collected in the Italian national surveillance system use self-report questionnaires, which can potentially overestimate prevalence rates compared to the European study, which used actigraph accelerometers that provide a more accurate reading.

Table 2 Results of the final linear regression model

	β	95% CI	p-value	R^2	Adjusted R^2 change	%variance[c]
Age	0.36	0.25, 0.47	< 0.001	0.29	-	-
Kidmed index[a]	0.07	0.01, 0.14	0.043	0.32	+ 0.03	+ 10.3%
Sport participation outside school hours				0.36	+ 0.04	+ 12.5%
No	—	—				
Yes	0.58	0.09, 1.1	0.021			
Father educational level				0.41	+ 0.07	+ 13.9%
Middle school or lower[b]	—	—				
High school	0.57	0.14, 1.0	0.009			
University degree or higher	0.79	0.30, 1.3	0.002			

One observation was eliminated due to missing data. Regression coefficients standardized. Adjusted $R^2 = 0.41$

CI confidence interval

[a]KIDMED derived from Zoom-8

[b]Reference (comparison) class

[c]From the base adjusted R^2

In our study, the results of the regression analysis showed that older children have greater memory capacity, suggesting that maturation plays a fundamental role in working memory functions [10, 24]. This finding is consistent with the developmental literature, which documents that age is quite relevant to WM, especially in samples younger than 12 years old [55, 56]. We also found that gender did not affect performance. Other studies have shown that puberty may mark the onset of developmental changes in cognitive performance [57]. Our sample is somewhat younger than the stage of puberty and this may have attenuated gender differences.

Our findings also comport with other studies that find relations between different types of diet and cognitive functioning [26, 58, 59]. The underlying relationship between dietary adherence and cognitive functioning can possibly be traced to different nutrient characteristics inherent in the MD diet. For instance, the MD is associated with higher intake of polyphenols compared to other diets, which have been shown to suppress cognitive decline [60]. Furthermore, steady and prolonged polyphenol intake is related to lower blood pressure and limits adiposity, and has also been associated with improvements in lipid profile, anti-inflammatory effects, and low levels of oxidative stress, all of which has been shown to improve cognitive function [61]. A healthy diet like the MD relates to higher levels of perceived well-being, which can help to encourage self-determined behaviors, reduce levels of anxiety, and improve self-regulation in stressful situations [62–64]. Conceivably, the benefit of the MD may underscore the value of omega-3 fatty acids, which has also been shown to be associated with improved cognitive function [65]. Moreover, according to a recent literature review [66], there is evidence that gut microbiota is linked to cognitive performance. There is a strong interaction between diet and gut microbiota [67], thus improving gut microbiota through diet could lead to better cognitive performance.

Contrary to other studies which investigated the effect of PA [68] and night sleep [69] on WM, our results showed that the PA data obtained from the accelerometer and the number of sleeping hours per night were not related to cognitive performance. However, engagement in organized sport was associated with better WM performance. This finding supports the growing body of research showing consistent positive relations between sport participation, cognition, and academic performance [4, 70–75]. Reinforcing these findings is a recent systematic review suggesting that organized sports might be a better option for developing executive functions compared to simply increasing PA [4]. In particular, other studies suggest that the main effect in WM performance is related to open-skill and closed-skill exercises, which is observed in older children (9–10 years) [76]. Moreover, higher physical fitness has been linked with better cognition and academic achievement in children [74], suggesting that sport and physical fitness are positively associated with important higher-order executive cognitive functions.

The effect of fathers' education on children's WM abilities is worth noting in light of the prominent role played by mothers in their child's development. Differences in the mean levels of mother's education were not significant (univariate analyses), and in fact, were ever so slight when comparing the reference group to the higher education levels. The lack of notable differences in educational levels may reflect the fact that all mothers are involved to some degree in their child's nutritional decisions, regardless of education. This is particularly true in Italy, where mothers play an integral role in food selection and preparation (i.e., from a culinary standpoint it is a matriarchal society). Other studies reinforce the central role played by mothers in their child's cognitive involvement, self-worth, and reading skills [77]. The bottom line is that the mean differences for educational levels were not significant for mothers while for fathers mean differences in WM for educational levels differed dramatically. In many cases, fathers may play an integral role in the development of their children, including building competence and supporting cognitive development. Although they traditionally spend less time with their children the quality of their time may be more important, especially if their time is devoted to engaging in activities (i.e., reading books at night or playing games that stimulate imagination) that promote WM growth. In light of this posited relationship, education may serve as a proxy for other parent–child relationship qualities including parent–child communication, and engagement in competence-building activities that can lead to expanded memory capabilities.

Other studies have also reported that parent education correlates with higher WM [16]. In many of these studies, children from low socioeconomic status homes have poorer educational outcomes than higher socioeconomic status [16, 78]. Specifically, high socioeconomic status is associated with better school performance and WM [79, 80]. Nevertheless, to the best of our knowledge, the present study is the first of its kind to report that fathers' educational level is an efficient predictor of children's WM. Considering the novelty of the association and the relatively small sample size of the present study, further investigations are needed to properly evaluate the impact of fathers' educational level on children's cognitive function.

This study has several limitations. First, the study population was relatively small and limited to a specific part of northern Italy. A larger sample would produce more stable parameter estimates and yield better representation of behavior. Conducting the study in different regions, even within Italy, would allow for differences in dietary practices that lead to different patterns of food consumption. This would also hold true for generalizing the study findings to a larger European and international audience. Second, we did not take into account the seasonality of lifestyle habits, which may also influence food consumption. Third, we did not investigate the full array of socioeconomic factors and family-based factors that can influence both diet and WM, suggesting that models can be mis-specified merely by the omission of relevant variables. The study was cross-sectional limiting causal inferences, particularly in a situation where WM can cause dietary adherence as much as dietary adherence can cause WM. Future longitudinal studies can help unravel this causal nexus and also examine developmental change in WM and factors that influence adherence. Physical activity and involvement in sport activities also changes with time, suggesting that there may be critical periods where instruction in diet, activity, and cognitive functioning may be best targeted. Finally, we relied on a restricted set of family-based and child variables as confounders in the regression models. Using a wider set of control measures would gain precision in the model but also enable us to eliminate variables that do not contribute unique variance. For instance, including measures of parent–child communication, parent–child relations, or time spent with children during the course of a week might clarify the underlying linkage between father's education and WM. Gaining clarity on the nature of these relations can help to reduce spurious relations that is notably present with cross-sectional studies.

Conclusions

The present study emphasizes the importance of lifestyle determinants that affect the WM of primary school children. Specifically, our data underline the need for interventions to improve children's adherence to the MD and encourage participation in organized sport activities that could be implemented in the school environment as the setting of choice. Furthermore, the results highlight that health promotion interventions based on PA and sound nutrition involving children benefit not only physical and mental health but also cognitive health.

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Authors' contributions A.M., F.S., and L.D. conceived the study. A.M., F.S., R.S., and L.D. contributed to the study design. A.M., F.S., and LS drafted the manuscript, which was integrated with important intellectual content by all authors. A.M., F.E., A.K., and S.S. collected and imputed the data. F.S. performed the statistical analysis. L.D., A.T., and R.S. supervised the study. The final manuscript was read and approved by all of the authors. All authors have read and agreed to the published version of the manuscript.

Data availability The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical and privacy reasons.

Declarations

Informed consent Informed consent was obtained from all subjects involved in the study.

Competing interests The authors declare no competing interests.

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