Maternal nut intake in pregnancy and child neuropsychological development up to 8 years old: A population-based cohort study in Spain

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Short running head

Maternal nut intake and child neurodevelopment

Abbreviations

ALA: Alpha-linolenic acid

ANT: Attention Network Test

BNDF: Brain-derived neurotrophic factor

BMI: Body mass index

BSID: Bayley Scales of Infant Development

CI: Confidence interval

DAG: Directed Acyclic Graph

DALYs: Disability adjusted life-years

DHA: Docosahexaenoic acid

EPA: Eicosapentaenoic acid

FDR: False discovery rate

FFQ: Food frequency questionnaire

FWER: Familywise error rate

HRT-SE: Hit Reaction Time Standard Error

IQ: Intelligence quotient

INMA: Infancia y Medio Ambiente [Environment and Childhood]

IQR: Interquartile range

IPW: Inverse probability weighting

MSCA: McCarthy Scales of Children's Abilities

PREDIMED: Prevention with Mediterranean Diet

PUFAs: Polyunsaturated fatty acids

rMED: Relative Mediterranean Diet Score

SD: Standard deviation

Abstract

There is scientific evidence on the protective effects of nut intake against cognitive decline in the elderly; however, this effect has been less explored in child neurodevelopment and no studies have explored the potential longitudinal association with nut intake during pregnancy. We aimed to analyze the association of maternal nut intake during pregnancy with child neuropsychological outcomes. We included 2208 mother-child pairs from a population-based birth cohort in four regions of Spain. The follow up settings were during pregnancy (first and third trimesters), birth, 1.5, 5 and 8 years. Neuropsychological examinations were based on Bayley Scales of Infant Development (1.5 years), McCarthy scales of Children's Abilities (5y), Attention Network Test (ANT, 8y) and N-Back test (8y). Nut intake in pregnancy was reported through a validated food frequency questionnaire during the first and the third trimester. Multivariable regressions analyzed associations after controlling for priori selected confounders notably maternal education, social class, body mass index, energy intake, fish intake, omega-3 supplements, alcohol consumption and smoking habits during pregnancy. Children within the highest tertile of maternal nut consumption during first pregnancy trimester (>32g/week) had an increase of 2.37 points (95% confidence interval [CI] = 0.76, 3.98) in the McCarthy global cognitive scale, compared to the first tertile (median 0g/w). A similar association pattern was observed with the other cognitive scores at the different child ages. Final model estimates by inverse probability weighting (IPW) did not change results. Third pregnancy trimester nut intake showed weaker associations. These data indicate that nut intake during early pregnancy is consistently associated with long-term child neuropsychological development. Future cohort studies and randomized clinical trials are needed to confirm this association pattern in order to further extend nutrition guidelines among pregnant women.

Keywords: Nut, maternal diet, children, neurodevelopment, population-based cohort.

Introduction

Nuts are composed of a matrix of nutrients containing a substantial amount of plant protein, vitamins, minerals and polyunsaturated fatty acids (PUFAs). Globally, in 2016, a diet low in nuts and seeds was considered as a leading dietary risk for disability-adjusted life years (DALYs)[1]. A significant bulk of research has been carried out on the cardiovascular and metabolic benefits of nuts such as decreased risk of hypertension, oxidative stress and diabetes [2, 3]; however their potential positive effects on the human brain have been only recently studied [4, 5]. Previous studies indicated that nuts act on several brain dysfunctions among aging adults and highlighted the need to further investigate nut's neuroprotective compounds and biological mechanisms. A randomized controlled trial focusing on Mediterranean diet supplemented with nuts observed a reduction of the age-related cognitive declining function among older population [6]. Other studies among older individuals having a diet pattern including nuts as an important component also showed a negative association with depression and mild cognitive disorders [7].

These beneficial associations could be attributed to the nut content of essential fatty acids or other nutritional components such as folic acid. Hence, if the brain can be influenced by those nutritional and biological factors late in life, it appears relevant to bring attention to another important time spanning that is early human life, including uterine period, where optimal brain development and function may require those key elements. Since the brain undergoes through a number of complex processes during human gestation, maternal nutrition appears to be an important factor contributing to an adequate fetal neurodevelopment with long-term effects [8, 9]. More specifically, cognitive function is responsible for critical skills such as attention, executive function and working memory [10]. Consequently, the fact that the brain is in constant development even well before the birth of the offspring, deficits of certain nutritional components in the diet of the pregnant woman may have long-term functional consequences on these numerous complex processes of the children [11]. Thus at present, the possible neurodevelopment benefits of maternal nut consumption for the child is an hypothesis that has not yet been explored in public health and epidemiology, indeed, considering the possibility of observing long-term associations with important cognitive endpoints during brain maturation in several childhood periods [12].

In the present study we assessed associations between pregnancy nut consumption and three longitudinal assessment settings of neuropsychological outcomes including child global cognitive functioning, attention and working memory in a Spanish multicenter cohort study.

Subjects and Methods

Study population

The Spanish Childhood and Environment (Infancia y Medio Ambiente, INMA) Project is the multicenter prospective birth cohort study included in this analysis. It was established in 4 regions of Spain between 2003 and 2008: Asturias, Gipuzkoa, Sabadell and Valencia. Participant recruitment and follow-up procedures have been reported in detail elsewhere [13]. A total of 2644 eligible pregnant women were recruited during prenatal visits in the first trimester of pregnancy corresponding to the inclusion criteria (≥ 16 years of age, singleton pregnancy, intention to deliver at the reference hospital) and exclusion criteria (communication handicap, fetuses with malformations, assisted conception) (Figure 1). Mothers were followed up during pregnancy, and their children were enrolled at birth and followed until the age of 8 years. After excluding women who withdrew, were lost to follow-up, underwent abortions or had fetal deaths, a total of 2498 pregnant women were monitored through delivery. Afterwards, children completed different neuropsychological tests up to the age of 8 years old. Final analyses included 2208 children around 1.5 year old, 1818 children around 5 years old and 1658 children around 8 years old (Figure 1). We excluded 18 children with pathologies including plagiocephaly. The remaining losses are attributable to follow up and missing data on some co-variables (Supplementary Methods 1). All participants provided written informed consent at recruitment and at each follow-up. The study protocol was approved by hospital and institutional ethics committees in each region. Further information is shown in Supplementary Methods 1.

Exposure and co-variable information

A semi-quantitative food frequency questionnaire (FFQ) of 101 food items was used to assess the daily intake of foods and nutrients during the first trimester of pregnancy (10-13 weeks) to estimate typical dietary intakes from preconception to the third month of pregnancy, and the third trimester of pregnancy (28-32 weeks) to estimate typical dietary intakes from the fourth to the seventh months of pregnancy. The FFQ was a modified version of a previous FFQ based on the Harvard questionnaire [14], that was adapted and validated for Spanish population [15]. Since most of the food composition tables in Spain were mainly based on bibliographic compilation and provided limited information for some nutrients, we used US Department of Agriculture food-composition tables [16] as well as a food composition table that included nutrient information for specific foods analyzed in Spain to obtain nutrient values [17, 18]. Women had to report their usual intake of foods from the last menstruation to the first prenatal visit and then again from first prenatal visit to third trimester visit, using reference portion sizes and nine frequency categories ranging from never/less than once a month to more than six times per day. The questionnaire included one item related to nut intake (including walnuts, almonds, peanuts, pine seeds, hazelnuts (1 portion of 30g). Thus, exposure variable of maternal nut intake during 1st trimester refers to the first three months of pregnancy and maternal nut intake during 3rd trimester refers to the 2nd trimester and first month of the 3rd trimester. Later, the responses were converted into weekly nut intake expressed in grams (g/week). In order to obtain a variable that represents an increment of consumption equivalent to a serving size, g/week consumption was divided by 30 (1 serving-size = 30g). In order to obtain categories of consumption, nut intake was further categorized into tertiles to obtain a category of low, medium and high intake. Fish consumption (g/week), Mediterranean diet score (rMED) and total energy intake (kcal/day) were estimated from the FFQ. Information on use of supplements, i.e. omega-3 fatty acid and folate was also gathered from questionnaires. Similar proceedings were applied to estimate child nut intake from a FFQ including 105 food items that was administered to mothers to estimate their child's usual daily intake of foods and nutrients at the age of 5 years. Information about the children's consumption was collected with 9 different intake frequencies from "never or < once a month" to "≥ 6 times per day". The FFQ was derived from an adult version of the FFQ that had previously been validated among the mothers of the children. A subsample of cord blood was obtained from the newborns at delivery [19]. PUFAs concentrations were then analyzed in cord plasma (n = 947) by fast-gas chromatography. Individual alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were measured and expressed in percentage of the total fatty acids. More details about how they were assessed can be found in the Supplementary Methods 2.

In order to obtain co-variable information, trained interviewers administered questionnaires to parents twice during pregnancy and at child ages of 1.5, 5 and 8 years. Questionnaires administered were used to obtain information on maternal characteristics such as pre-pregnancy body mass index (BMI, kg/m^2), age, education level, occupational social class, country of birth and smoking habits throughout pregnancy. Gender of the child and birth weight, were recorded by trained midwives at delivery. More details about how they were assessed, including other co-variables for secondary analyses, can be found in the Supplementary Methods 2.

Neuropsychological assessments

Children were assessed by internationally validated and standardized neuropsychological scales and computer-based tests up to the 8-year old visit. The neuropsychological scales used for measuring global motor and cognition outcomes were the Bayley Scales of Infant Development (BSID) at child mean age (Standard deviation, SD) of 1.67 (0.22) years old, and the McCarthy Scales of Children's Abilities (MSCA) at child age of 4.84 (0.62) years old. At child age of 7.59 (0.62) years old, children were assessed with two computer-based tests, the Attention Network Test (ANT) to assess attention function (executive function and hit reaction time standard error (HRT-SE)) and the N-Back test to assess working memory (N-Back – Detectability number 1-back). ANT outcomes are inversed meaning that the higher scores indicate a lower performance of the child. Further information about scale and test characteristics, extended list of outcomes not shown in the main tables, and examination proceedings are shown in Supplementary Methods 3.

Statistical analysis

Associations between maternal nut consumption during pregnancy and child neuropsychological outcomes were evaluated using separate multivariable linear regression analyses. Multivariable censored regression (tobit) was used to assess the association between maternal nut consumption and N-Back detectability n-1.Nut consumption was adjusted for energy intake using the residual method [20]. Nut intake was evaluated both as continuous (per 30 g/week increments) and as ordinal (in tertiles of weekly grams, first tertile as the reference category) variables. Since diet is measured with some degree of error and therefore measured intake is unlikely to represent true intake accurately [21], we categorized dietary data and assume that those in the extremes of consumption are likely to truly have low and high intakes of that specific food (nuts, in this case). Tests for linear trend were performed by including median values of consumption within each tertile category in the regression models. Spearman rank correlation coefficient was calculated between maternal nut intake during 1st trimester and maternal nut intake during 3rd trimester as continuous variables. Minimally-adjusted regression models included adjustment for the age and gender of the child, cohort location, total maternal energy intake (kcal), and quality of the test performance flagged by the psychologist (good versus not-so-good; BSID and MSCA only). The final models were additionally adjusted for child's weight at birth, maternal education (primary school or less, secondary school, university or more), maternal social class (highly skilled, non-manual, manual), maternal body mass index (BMI) based on measured height at recruitment and pre-pregnancy self-reported weight, maternal fish intake in grams per week, omega-3 supplements, alcohol consumption (yes/no) and smoking habits (yes/no) during pregnancy. Confounders were selected using a Directed Acyclic Graph (DAG) model as illustrated in (Supplementary Figure 1) [22]. Additional models stratified by socioeconomic status and maternal education were run in order to verify potential effect modification by these variables and rule out residual confounding. Moreover, sensitivity analyses were performed using maternal verbal intelligence quotient (IQ) proxy (WAIS-IV Similarities subtest) [23], maternal psychopathological symptoms (SCL-90-R) [24-25], maternal country of birth, paternal social class, maternal folate supplement intake and the relative Mediterranean diet score [26] as confounders, as well as child breastfeeding duration, child nut intake and family's urban vulnerability index [27] during pregnancy. Final models were repeated with maternal nut intake at the third trimester of pregnancy. However, in the secondary analyses, we put more focus on first pregnancy trimester due to observe stronger associations.

Bonferroni family wise error rate (FWER), Hochberg FWER and Simes false discovery rate (FDR) corrections were applied for multiple testing in secondary analyses.

In order to further explore potential risk for residual confounding, we assessed the association between maternal nut intake during first trimester of pregnancy and maternal social class and education using ordered logistic regression (proportional odds model) and repeated the final models by cohort location. Furthermore, change-in estimates method was used in order to determine the estimate percentage change due to the co-variables included in the final models of the associations between nut intake and the main cognitive outcomes.

In order to correct for the potential selection bias due to the loss of observations, we applied the Inverse Probability Weighting (IPW) [28]. We used information available for all participants at recruitment to predict the probability of participation in the study and used the inverse of those probabilities as weights in the analysis. All analyses were conducted with STATA 12 statistical software package and statistical significance was defined as having a p-value < 0.05.

Results

The mean nut consumption among mothers in the first trimester of pregnancy was 41 g/week (SD 74 g/week) and the median was 17 g/week (IQR = 0; 46.33 g/week). A third of the total participants were non-consumers (n=860, 33.50%). Spearman correlation coefficient between maternal nut intake in the first trimester and maternal nut intake in the third trimester was moderate (r=0.39, p-value < 0.01). Nut intake was higher among mothers with higher verbal IQ proxy, education and social class, lower BMI, were born in Spain, did not smoke tobacco and did not consume alcohol during pregnancy. Mothers who reported low energy intake (in kilocalories) and scored higher in the Mediterranean diet, also showed a higher nut intake. In terms of the child characteristics, the higher the maternal nut intake, the higher duration of breastfeeding and the higher the umbilical cord blood levels of EPA and DHA (Table 1). The highest Spearman correlation coefficient among nut intake and PUFAs was of DHA concentration (r=0.16; p-value: < 0.01). In terms of paternal characteristics, higher maternal nut intake was associated with fathers who were born in Spain and highly skilled (Supplementary Table 1). Child neuropsychological scores by maternal nut consumption in both trimesters are shown in Supplementary Table 2 and Supplementary Table 3.

Minimally and fully adjusted associations of maternal nut consumption during first trimester and third trimester of pregnancy with the main child neuropsychological outcomes up to 5 years old are shown in Table 2. Extended associations with all neuropsychological outcomes assessed are shown in Supplementary Table 4. During the first trimester of pregnancy, most of the outcomes were independently associated with the exposure in both minimally and fully adjusted models. Associations between maternal nut intake and child BSID scores were positive, in both continuous (per 30g/week increase) and categorical (in tertiles) nut variable models. A BSID mental increment (β) of 1.86 (95% Confidence Interval (CI): 0.45, 3.27) points was observed for the highest tertile of nut consumption compared with the lowest tertile in the fully adjusted model, and a significant linear trend across nut intake tertiles (P for trend of 0.02) was observed. At age 5 years, children born of mothers in the highest tertile of nut consumption had a MSCA global cognitive score of 2.37 (95% CI: 0.76, 3.98) higher than the reference group in the fully adjusted model and with a p for trend<0.01. Similar results were observed for MSCA executive function scores.

Table 3 shows the associations between maternal pregnancy nut consumption (both trimesters) and 8-year-old child scores on different neuropsychological tests. Significant associations between first trimester nut consumption (tertile 3 vs tertile 1) and improvements in the ANT executive attention scores (β= -13.36, 95% CI: -23.31, -3,41), Hit Reaction Time-Standard Error (HRT-SE in milliseconds) (-13.82, 95% CI: -23.40, -4.23) and N-Back's detectability number-1-back (0.33, 95% CI: 0.01, 0.65) were observed. The results barely changed between minimally and fully adjusted models. Extended associations with all neuropsychological outcomes assessed are shown in Supplementary Table 5. Both in Table 2 and Table 3 models, the nut intake during the third trimester of pregnancy appeared to show weaker findings than the previous ones with the first trimester period.

When final models of Tables 2 and 3 were repeated, no interactions by maternal socioeconomic status and education level were observed (p-values for interactions > 0.05). The coefficients were not substantially different when stratifying the final models by maternal social class (Supplementary Table 6).

The exclusion of potential outliers (mothers with very high amount of nut consumption) and the adjustment for folate supplements and maternal country of birth, did not change overall results (data not shown), and additional adjustment for maternal IQ proxy, psychopathological symptoms, relative Mediterranean diet score, child nut intake, breastfeeding, paternal social class and the family's urban vulnerability index did not change results (Supplementary Table 7). Further saturating the models for all the co-variables presented in Supplementary Table 1 did not change the previous results (Supplementary Table 8). Additionally, IPW estimates did not change overall results (Supplementary Table 9).

Maternal social class and education level were similarly associated with maternal nut intake by cohort location (Supplementary Table 10). Most of the first pregnancy trimester nut intake and child cognitive outcome associations were similar by cohort location. However, Valencia location showed weaker associations with Bailey and MCSA outcomes but not between maternal social class and education and nut intake. 8-year-old ANT HRT-SE showed the most consistent results with low coefficient reductions between minimally and fully adjusted coefficients and by cohort locations, including Valencia. Moreover, after applying change-in estimates method in pooled analyses with the main cognitive outcomes, the highest percentage change of the association between maternal nut intake and BSID mental score appeared to be the variable maternal social class with -12.35% (Supplementary Table 11). Yet, for both associations with MSCA global cognitive and ANT-HRT-SE, the confounding variable inducing the highest percentage change was maternal education, with -26.37% and -13.81%, respectively.

To correct for multiple statistical comparisons, FWER and FDR p values are shown in Supplementary Table 12. Pre-natal nut intake during first trimester of pregnancy was associated with different neurodevelopment outcomes such as ANT Hit reaction time SE, MSCA global cognitive, MSCA executive function and BSID mental score (uncorrected P-value < 0.05). With FWER-correction ANT- Hit reaction time SE was significant. By FDR-procedure, ANT- Hit reaction time SE association was also significant, however, with this latter method, the other above mentioned outcomes showed corrected p-values < 0.07.

Discussion

In this longitudinal cohort study, we found that higher maternal intake of nuts in early pregnancy was associated with enhanced neuropsychological development in offspring at 1.5, 5 and 8 years old. The significant associations are observed in those in the highest tertile of nut intake, with about 3 servings per week (average), an amount slightly lower but within the recommended range by the current Spanish nutritional guidelines, which is between 3 and 7 handful servings of nuts per week, each serving is about 25 grams of nuts [29]. These findings are important to consider since nuts are regularly consumed in European diets, but average consumption in the population is still lower than the recommendation. In Spain, the mean intake of total nuts was estimated to be 4.8g per day (5.8g in our sample) compared to 2.2 per day for the entire European population [30]. In United States, 38.2% of the adult population report to consume nuts on a given day [31].

To the best of our knowledge, no previous studies assessed the relation between maternal nut intake in pregnancy and neuropsychological functioning in the offspring. A cohort study of 317 Korean children with a mean (SD) age of 11.8 (3.3) years conducted in 2014 found that the child consumption of nuts showed a positive cross-sectional correlation with cognitive reaction time consistency [32]. The test used was the symbol digit modality, which has shown significant correlations with some measures of the ANT, such as present study's Hit Reaction Time –Standard Error (HRT-SE) [33].

Nonetheless, several studies evaluating the association of nut intake and neuropsychological outcomes were identified, in particular among adults of advanced age. Many of the neuropsychological functions assessed were similar to the ones included in the INMA cohort study, such as working memory and executive function [5, 34-36]. For instance, the Prevention with Mediterranean Diet (PREDIMED) study conducted a large randomized cardiovascular prevention trial in Spain and evaluated the effect of the Mediterranean diet supplemented with 30 g/day of raw and unprocessed mixed nuts (15 g of walnuts, 7.5 g of almonds and 7.5 g of hazelnuts) versus the recommended low-fat diet for cardiovascular disease prevention [34]. Results indicated that the participants (from 55 to 80 years old) with a supplement of mixed nuts had higher scores on Mini-Mental State Examination and the Clock Drawing Test than the low-fat control group, indicating an improved cognitive functioning. Similar results were reported in another parallel-group study, which followed the structure of this PREDIMED protocol where follow-up cognitive tests were given with four cognitive composites: memory, global cognition, attention and executive function to 447 older adults (mean age of 66.9 years) [6]. The participants with a supplement of mixed nuts showed improvement on the memory index, relative to the baseline scores, and improved cognitive function when they were compared with the control group. Finally, in a randomized, placebo-controlled, crossover trial, 64 participants of 18 to 25 years old were asked to consume either banana bread with or without walnuts (60g/day) for 8 weeks with a 6-week washout period. After consuming banana bread with walnuts, participants showed improvement in inferential reasoning scores on the Watson-Glaser Critical Thinking Appraisal compared to those who ate the banana bread without nuts [37]. Further interventional studies should be done in children and pregnant women when neurodevelopment is highly active [12].

An explanation for this association that could be put forward resides in the nut's nutrient composition. In the Spanish population, it is estimated that nut consumption includes mostly almonds and walnuts [38], the latter being main nut source of ALA. During pregnancy, part of ALA is transformed to brain needed DHA and EPA [39]. Studies showed that ALA can improve learning and memory and ALA supplements was shown to enhance brain plasticity and to increase levels of brain-derived neurotrophic factor (BDNF), which is a widely distributed protein in the brain carrying out an important number of functions such as neuronal maintenance and neurogenesis [40, 41]. Moreover, during pregnancy, important amount of DHA is transferred across the placenta to the fetus [42]. During the foetal development, DHA tend to accumulate in neural tissues, firstly in the frontal areas of the brain which impact memory and executive functions [43]. Anti-inflammatory activity induced by nuts nutrients such as PUFAs is also a fundamental mechanism that could improve cognitive function since studies have shown that same property to have cerebrovascular protective effects among adults [44].

Furthermore, the associative patterns of this study tended to show similar results after applying several statistical analyses, such as stratifying the regression models by cohort location and maternal social class, and after adjusting the pooled analyses for a wider range of co-variables including maternal IQ proxy and mental health. In relation to the stratified results by cohort location, only Valencia, 1 out of 4 locations, showed weaker associations with Bailey and MCSA outcomes but similar association patterns with maternal education and social class, this latter finding indicates other unknown cohort location factors may be involved in these reduced coefficients. However, ANT-HRT-SE showed very consistent results with small coefficient changes within all the sensitivity analyses tested here. As expected, given that healthy dietary habits correlate with education in most epidemiological studies [45], maternal social class and education explained a higher degree of confounding variability in the multivariable regression analyses with change-in estimates method, but with low percentage changes (below 15 percent decreases) in two out of three main cognitive outcomes, except for MCSA global cognitive with a significant 26 percent decrease. Generally, all these above described findings indicate a reduced certainty for residual confounding by maternal social class and education, particularly with 8-year-old ANT-HRT-SE outcome, a highly related neurobiological measurement [46], however we cannot rule out this risk in observational studies such this one and randomized controlled trials are needed to confirm our findings. The main strengths of this study include the population-based prospective design with recruitment during pregnancy and relatively long-term follow-ups, and a large sample size from a multicenter cohort established in different geographical regions of Spain, and with identical assessment protocols. In addition, a strict protocol applied for the neuropsychological assessment on the children helped to improve the psychometric characteristics of the outcomes and ensured the quality of the results. Also, the consistency of the results in relation to the extensive sensitivity analyses applied here reinforced our conclusions, and the reliability of the beneficial associations observed repeatedly across the three settings of neuropsychological assessments during follow up, indicated a good predictive validity of the findings.

The study faced some limitations. The FFQ, despite being a validated tool, is subjected to measurement errors that may lead to the attenuation of the effect estimate. About 50% of the women who were approached for recruitment participated in the study, with a slight tendency for lower socioeconomic status among refusals. Because of this, extrapolation of the results to the general population requires caution. Furthermore, although the risk for residual confounding cannot be ruled out due to the observational nature of the study design, a wide range of potential confounders has been carefully considered using DAG modeling and evidence-based literature review, and we additionally conducted sensitivity analyses in order to address this potential limitation and others such inverse probability weighting estimates to control for both selection bias and bias due to missing data. It is true that, in this study, as in other nutritional epidemiology studies, effect sizes can be small to be clinically meaningful. However, in this project we were interested in studying possible associations that were not reported previously. The effect size can be attenuated due to the measurement error associated to dietary assessment tools. It has been estimated that true effect size would be larger if diet was not measured with error [47]. Finally, the level of nut consumption is relatively low but similar to other observational studies that found health beneficial associations [48, 49].

Overall, the present study suggests that nut intake during the first trimester of pregnancy is consistently associated with long-term child neuropsychological development. Further studies, such as randomized controlled trials, are necessary to disentangle the link between maternal nut consumption on the developing brain and the benefits on neuropsychological functioning in childhood. This line of work could help to support and improve the current dietary guidelines for pregnant women for optimal child neurodevelopment.

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Table 1: Baseline characteristics of the study participants according to tertiles of maternal nut consumption in the first trimester of pregnancy. Spanish Childhood and Environment (INMA) Project, 2004–2008.

	Tertiles of Maternal Nut Intake[e]			
	Low (n=860)[c]	**Medium (n=852)[c]**	**High (n=855)[c]**	**P-Values[d]**
Maternal characteristics				
Nut intake in g/week, median	0	17.52	73.94	
(rank)	(0, 0.56)	(1.78, 32.35)	(32.36, 920.23)	
(IQR)	(0.001, 0.004)	(11.68, 24.27)	(46.31, 123.95)	
Age in years, mean (SD)	30.34 (4.55)	30.59 (4.35)	30.83 (4.22)	0.07
Cohort location, *n* (%)[a]				
Asturias	189 (22)	127 (15)	165 (19)	< 0.01
Gipuzkoa	165 (19)	167 (20)	287 (34)	
Sabadell	238 (28)	221 (26)	194 (23)	
Valencia	268 (31)	337 (40)	209 (24)	
Pre-pregnancy BMI in kg/m^2, mean (SD)	24.44 (4.83)	23.44 (4.41)	22.86 (3.49)	< 0.01
Verbal IQ[b], mean (SD)	9.64 (2.99)	10.02 (2.98)	10.13 (2.93)	0.02
Education, *n* (%)[a]				
Primary school or less	245 (29)	234 (28)	167 (20)	<0.01
Secondary school	358 (42)	368 (43)	333 (39)	
University or more	255 (30)	249 (29)	354 (41)	
Social class, *n* (%)[a]				
Highly skilled	142 (17)	161 (19)	245 (29)	< 0.01
Non-manual	221 (26)	208 (24)	231 (27)	
Manual	497 (58)	483 (57)	378 (44)	
Country of birth, *n* (%)[a]				
Spain	765 (89)	778 (92)	804 (94)	< 0.01
Latin America	70 (8)	43 (5)	29 (3)	
Other European countries	20 (2)	23 (3)	15 (2)	
Other	4 (1)	6 (1)	7 (1)	
Smoking during pregnancy, *n* (%)[a]				
Yes	159 (20)	165 (20)	118 (15)	0.01
No	647 (80)	641 (79)	682 (85)	
Alcohol consumption during first trimester of pregnancy, *n* (%)[a]				
Yes	42 (5)	89 (10)	67 (8)	< 0.01
No	818 (95)	763 (90)	788 (92)	
rMED, mean (SD)	8.02 (2.65)	7.66 (2.51)	8.31 (2.61)	< 0.01
Energy intake in kcals/day during first trimester of pregnancy, mean (SD)	2023.33 (522.68)	2350.02 (581.4)	1987.02 (491.15)	< 0.01
Fish intake in g/day, mean (SD)	69.58 (41.75)	70.27 (39.15)	70.80 (37.49)	0.81
Omega-3 from supplements during first trimester of pregnancy, *n* (%)[a]				
Yes	17 (2)	33 (4)	22 (3)	0.42
No	823 (96)	806 (95)	807 (94)	

Abbreviations: BMI, Body mass index; IQ: Intelligence Quotient; IQR, Interquartile range; rMED: Relative Mediterranean Diet Score; SD: standard deviation. [a] Percentages are rounded to nearest number. [b] Similarities subtest of the Wechsler Adult Intelligence Scales, 3rd edition. [c] Some of totals do not match the total number of subjects because values were missing in some categories. [d] ANOVA p-value for continuous variables and Chi-square p-value for categorical variables. [e] Similar characteristics of the study participants were observed with tertiles of maternal nut intake during third trimester (data not shown).

Table 1 (continued): Baseline characteristics of the study participants according to tertiles of maternal nut consumption in the first trimester of pregnancy. Spanish Childhood and Environment (INMA) Project, 2004–2008.

	Tertiles of Maternal Nut Intake[e]			
	Low (n=860)[c]	**Medium (n=852)**[c]	**High (n=855)**[c]	**P-Values**[d]
Child characteristics				
Sex, *n* (%)[a]				
Male	439 (53)	413 (50)	416 (51)	0.51
Female	388 (47)	405 (50)	403 (49)	
Breastfeeding, *n* (%)[a]				
None	139 (18)	105 (14)	93 (12)	< 0.01
0-16 wks	219 (29)	186 (25)	172 (23)	
16-24 wks	120 (16)	110 (15)	128 (17)	
24 wks	296 (38)	345 (46)	362 (48)	
Birth weight in g, *n* (%)[a]				
< 3000	215 (26)	211 (26)	208 (26)	0.50
3000-3500	374 (45)	392 (48)	360 (44)	
< 3500	234 (28)	213 (26)	242 (30)	
Omega-3 fatty acid umbilical cord blood levels				
ALA in %, mean (SD)	0.11 (0.12)	0.10 (0.09)	0.09 (0.09)	< 0.01
EPA in %, mean (SD)	0.22 (0.19)	0.22 (0.14)	0.27 (0.21)	< 0.01
DHA in %, mean (SD)	4.86 (1.52)	5.00 (1.62)	5.43 (1.75)	< 0.01

Abbreviations: ALA, Alpha linolenic acid; BMI, Body mass index; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; IQ: Intelligence Quotient; IQR, Interquartile range; rMED: Relative Mediterranean Diet Score; SD: standard deviation

[a] Percentages are rounded to nearest number.

[b] Similarities subtest of the Wechsler Adult Intelligence Scales, 3rd edition.

[c] Some of totals do not match the total number of subjects because values were missing in some categories.

[d] ANOVA p-value for continuous variables and Chi-square p-value for categorical variables.

[e] Similar characteristics of the study participants were observed with tertiles of maternal nut intake during third trimester (data not shown).

Table 2: Multivariable regression analysis I: Associations between maternal nut consumption in the first trimester and in the third trimester of pregnancy and child's scores on BSID at 1.5 years old and MSCA at 5 years old, Spanish Childhood and Environment (INMA) Project, 2004–2016.

			Difference in Child's Neuropsychological Score					
			Minimally Adjusted[a]			**Fully Adjusted[b]**		
Neuropsychological outcome	**Maternal nut intake 1st trimester**	**No. of Subjects**	**β**	**(95% CI)**	**P for trend**	**β**	**(95% CI)**	**P for trend**
BSID – Mental score at 1.5 year	Per 30g/week increase	1985	0.53	(0.30, 0.77)[c]	-	0.48	(0.23, 0.72)[c]	-
	Lowest tertile	665	Ref.			Ref.		
	Middle tertile	658	1.63	(0.21, 3.05)[c]	<0.01	1.28	(-0.15, 2.71)	0.02
	Highest tertile	662	2.49	(1.11, 3.86)[c]		1.86	(0.45, 3.27)[c]	
MSCA – Global cognitive score at 5 years	Per 30g/week increase	1659	0.60	(0.33, 0.86)[c]	-	0.47	(0.21, 0.73)[c]	-
	Lowest tertile	545	Ref.			Ref.		
	Middle tertile	549	0.57	(-1.07, 2.22)	<0.01	0.51	(-1.15, 2.16)	<0.01
	Highest tertile	565	3.67	(2.04, 5.30)[c]		2.37	(0.76, 3.98)[c]	
MSCA – Executive function score at 5 years	Per 30g/week increase	1659	0.65	(0.38, 0.92)[c]	-	0.52	(0.26, 0.79)[c]	-
	Lowest tertile	545	Ref.			Ref.		
	Middle tertile	549	0.90	(-0.84, 2.63)	<0.01	0.22	(-1.48, 1.93)	0.01
	Highest tertile	565	3.50	(1.83, 5.16)[c]		2.18	(0.52, 3.83)[c]	
	Maternal nut intake 3rd trimester							
BSID – Mental score at 1.5 year	Per 30g/week increase	1990	0.13	(-0.12, 0.39)	-	0.07	(-0.18, 0.33)	-
	Lowest tertile	701	Ref.			Ref.		
	Middle tertile	654	0.17	(-1.27, 1.62)	0.39	-0.30	(-1.75, 1.15)	0.97
	Highest tertile	635	0.61	(-0.79, 2.01)		-0.04	(-1.45, 1.38)	
MSCA – Global cognitive score at 5 years	Per 30g/week increase	1657	0.31	(0.02, 0.60)[c]	-	0.18	(-0.10, 0.46)	-
	Lowest tertile	580	Ref.			Ref.		
	Middle tertile	532	1.24	(-0.50, 2.97)	<0.01	0.15	(-1.53, 1.83)	0.10
	Highest tertile	545	2.69	(1.02, 4.35)[c]		1.28	(-0.34, 2.90)	
MSCA – Executive function score at 5 years	Per 30g/week increase	1657	0.30	(0.01, 0.60)	-	0.17	(-0.12, 0.45)	-
	Lowest tertile	580	Ref.			Ref.		
	Middle tertile	532	1.15	(-0.62, 2.93)	<0.01	0.03	(-1.70, 1.76)	0.17
	Highest tertile	545	2.48	(0.78, 4.18)[c]		1.06	(-0.60, 2.73)	

Abbreviations: BSID, Bayley Scales of Infant Development; CI: Confidence Interval; MSCA, McCarthy Scales of Children's Abilities; Ref, Reference group.

[a] Beta coefficients and 95% CI estimated using linear regression models adjusted for sex of the child, child's age at testing, cohort location, quality of the test, and maternal energy intake (kcal/day) during pregnancy.

[b] Beta coefficients and 95% CI estimated using linear regression models additionally adjusted for maternal alcohol consumption, maternal education, fish intake, maternal smoking, maternal consumption of omega-3 fatty acid supplements, maternal BMI, maternal social class and child's birth weight.

[c] $P < 0.05$.

Table 3: Multivariable regression analysis II: Associations between maternal nut consumption in the first trimester and the third trimester of pregnancy and child's scores on ANT and N-Back at 8 years old, Spanish Childhood and Environment (INMA) Project, 2004–2016.

			Difference in Child's Neuropsychological Score					
			Minimally Adjusted[a]			Fully Adjusted[b]		
Neuropsychological outcome	**Maternal nut intake 1st trimester**	**No. of Subjects**	**β**	**(95% CI)**	**P for trend**	**β**	**(95% CI)**	**P for trend**
ANT – Executive attention (conflict) (ms)[c]	Per 30g/week increase	1591	-0.69	(-2.27, 0.89)	-	-0.30	(-1.93, 1.34)	-
	Lowest tertile	524	Ref.			Ref.		
	Middle tertile	519	-4.53	(-14.63, 5.56)	< 0.01	-5.22	(-15.51, 5.08)	0.01
	Highest tertile	548	-14.57	(-24.15, -4.98)[d]		-13.36	(-23.31, -3.41)[d]	
ANT – Hit reaction time SE (ms)[c]	Per 30g/week increase	1591	-1.70	(-3.22, -0.18)[d]	-	-1.28	(-2.86, 0.30)	-
	Lowest tertile	524	Ref.			Ref.		
	Middle tertile	519	0.39	(-9.33, 10.12)	< 0.01	2.16	(-7.76, 12.07)	< 0.01
	Highest tertile	548	-16.05	(-25.29, -6.81)[d]		-13.82	(-23.40, -4.23)[d]	
N-Back – Detectability number 1-back	Per 30g/week increase	1570	0.04	(-0.02, 0.09)	-	0.03	(-0.03, 0.09)	-
	Lowest tertile	515	Ref.			Ref.		
	Middle tertile	516	0.02	(-0.30, 0.33)	0.01	-0.14	(-0.33, 0.30)	0.02
	Highest tertile	539	0.40	(0.09, 0.71)[d]		0.33	(0.01, 0.65)[d]	
	Maternal nut intake 3rd trimester							
ANT – Executive attention (conflict) (ms)[c]	Per 30g/week increase	1453	-0.13	(-1.88, 1.61)	-	0.14	(-1.62, 1.90)	-
	Lowest tertile	507	Ref.			Ref.		
	Middle tertile	461	6.10	(-4.19, 16.39)	0.16	8.51	(-1.90, 18.91)	0.27
	Highest tertile	485	-5.17	(-14.97, 4.62)		-2.67	(-12.61, 7.28)	
ANT – Hit reaction time SE (ms)[c]	Per 30g/week increase	1453	0.60	(-1.08, 2.29)	-	0.83	(-0.87, 2.52)	-
	Lowest tertile	507	Ref.			Ref.		
	Middle tertile	461	-5.12	(-15.05, 4.83)	0.66	-2.47	(-12.49, 7.56)	0.91
	Highest tertile	485	-3.09	(-12.55, 6.38)		-0.98	(-10.56, 8.61)	
N-Back – Detectability number 1-back	Per 30g/week increase	1434	0.04	(-0.02, 0.09)	-	0.03	(-0.03, 0.09)	-
	Lowest tertile	502	Ref.			Ref.		
	Middle tertile	459	-0.01	(-0.46, 0.18)	0.52	-0.15	(-0.47, 0.16)	0.98
	Highest tertile	473	0.07	(-0.25, 0.38)		0.02	(-0.30, 0.34)	

Abbreviations: ANT, Attention Network Test; CI, Confidence Interval; Ref, Reference group.
[a] Beta coefficients and 95% CI estimated using linear regression models adjusted for sex of the child, child's age at testing, cohort, and maternal energy intake (kcal/day) during pregnancy. Tobit regression model was used for N-Back n1.
[b] Beta coefficients and 95% CI estimated using linear regression models additionally adjusted for child's birth weight, maternal alcohol consumption, education, fish intake, smoking, consumption of omega-3 fatty acid supplements, maternal BMI and social class. Tobit regression model was used for N-Back n1.
[c] The tests are inversed meaning that the higher scores indicate a lower performance of the child.
[d] $P < 0.05$.