

Mediterranean dietary pattern in pregnant women and offspring risk of overweight and abdominal obesity in early childhood: the INMA birth cohort study

Authors:

Sílvia Fernández-Barrés^{1,2} / Dora Romaguera^{1,3,4} / Damaskini Valvi^{1,5,6,7} / David Martínez^{1,5,6} / Jesús Vioque^{5,8} / Eva María Navarrete-Muñoz^{5,8} / Pilar Amiano^{5,9} / Sandra Gonzalez-Palacios^{5,8} / Mònica Guxens^{1,5,6,10} / Carmen Iñiguez¹⁴ / Eva Pereda¹¹ / Isolina Riaño^{5,12} / Adonina Tardón^{5,13} / Victoria Arija² / Jordi Sunyer^{1,5,6,15} / Martine Vrijheid^{1,5,6,15} on behalf of the INMA Project

Affiliations:

¹Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain

²Nutrition and Mental Health Group, Universitat Rovira I Virgili (URV), Reus, Spain

³Instituto de Investigación Sanitaria de Palma (IdISPa), Hospital Universitari Son Espases, Palma de Mallorca, Spain

⁴CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Madrid, Spain

⁵CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

⁶Universitat Pompeu Fabra (UPF), Barcelona, Spain

⁷Harvard T.H. Chan School of Public Health, Boston, Massachusetts, United States

⁸Universidad Miguel Hernandez, Alicante, Spain

⁹Public Health Division of Gipuzkoa, BioDonostia Research Institute, Spain

¹⁰Department of Child and Adolescent Psychiatry/Psychology, Erasmus University Medical Centre–Sophia Children’s Hospital, Rotterdam, The Netherlands

¹¹Facultad de Psicología, Universidad del País Vasco-Euskal Herriko Unibertsitatea (UPV-EHU), Spain

¹²Hospital San Agustín, SESPA, Asturias, Spain

¹³Universidad de Oviedo, Asturias, Spain

¹⁴FISABIO – Universitat Jaume I – Universitat de València Epidemiology and Environmental Health Joint Research Unit, Valencia, Spain

¹⁵Institut Hospital del Mar d'Investigacions Mèdiques-Parc de Salut Mar, Barcelona, Spain

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Address correspondence to **Dora Romaguera**, Instituto de Investigación Sanitaria de Palma (IdISPa), Hospital Universitari Son Espases. Edificio S, Carretera de Valldemossa, 79. 07120 Palma de Mallorca, Spain. E-mail: mariaadoracion.romaguera@ssib.es

What is already known about this subject:

- High maternal diet quality during pregnancy has been suggested as a protective factor to the development of obesity in the offspring.
- However, evidence from human studies is limited, and most previous studies have focussed on the macronutrient composition of the diet, with inconsistent results.

What this study adds

- Adherence to the Mediterranean diet during pregnancy was not associated with risk of overweight in 4 years-old children age in a large sample of mothers and children in Spain.
- However, adherence to the Mediterranean diet was associated with lower waist circumference, a marker of abdominal obesity.

ABSTRACT

Background: Animal models have suggested that maternal diet quality may reduce offspring obesity risk regardless of maternal body weight; however, evidence from human studies is scarce.

Objective: To evaluate associations between adherence to the Mediterranean diet (MD) during pregnancy and childhood general and abdominal obesity risk at 4 years of age.

Methods: We analyzed 1,827 mother-child pairs from the Spanish INMA cohort study, recruited between 2003 and 2008. Diet was assessed during pregnancy using a food frequency questionnaire and MD adherence by the relative Mediterranean diet Score (rMED).

Overweight (including obesity) was defined as an age- and sex-specific body mass index $\geq 85^{\text{th}}$ percentile (WHO referent), and abdominal obesity as a waist circumference (WC) $> 90^{\text{th}}$ percentile. Multivariate adjusted linear and logistic regression models were used to evaluate associations between pregnancy rMED and offspring overweight and abdominal obesity.

Results: There was no association between rMED and body mass index z-score, whereas there was a significant association between higher adherence to MD and lower WC (β of high versus low rMED: -0.62 cm; 95% CI: -1.10, -0.14 cm, P for trend = 0.009).

Conclusions: Pregnancy adherence to the MD was not associated with childhood overweight risk, but it was associated with lower WC, a marker of abdominal obesity.

INTRODUCTION

The current childhood obesity epidemic is a major problem of public health. Overweight and obese children are more likely to have obesity and other metabolic diseases later in life, thus early prevention is critical¹.

Several early life environmental factors have been associated with increased childhood obesity risk, such as pregnancy weight gain, birth weight, rapid postnatal growth and gestational diabetes^{2,3}. In contrast, preventive factors are breastfeeding and late solids introduction. Maternal overweight is an important predictor of childhood obesity⁴. What it is not yet clear is whether maternal diet in pregnancy has an effect on childhood obesity risk beyond maternal weight status. Some animal models have suggested that high quality maternal diet may reduce childhood obesity risk regardless of maternal body weight⁵; however, other studies have concluded that maternal diet itself, in the absence of obesity is insufficient to predispose offspring to obesity; maternal obesity is required for this programming effect⁶. The mechanisms of action are uncertain; suggested mechanisms include the glycemic effect of certain diets with a high glycemic index which lead to hyperglycaemia *in utero* and foetal hyper-insulinemia, which may influence individual susceptibility to weight gain later in life⁷. On the other hand, maternal obesity and high-fat intake in pregnancy may induce placental inflammation, and thus increases nutrient transport and foetal growth. Finally, western diet in pregnancy and lactation has been associated with leptin insensitivity in the offspring, leading to hyperphagia and increased weight gain⁵. Only few studies have evaluated the role of maternal diet on childhood obesity risk in humans. Most studies have focussed on the macronutrient composition of the diet and results have been mostly inconsistent⁷⁻¹⁰. The study of dietary patterns represents a broader picture of dietary intake and may be more useful to assess the diet quality and to predict disease risk¹¹.

The Mediterranean diet (MD) is considered a healthy dietary pattern and has been associated with lower obesity development risk in both children¹² and adults¹³. Recent studies have suggested that a lower maternal adherence to a MD in pregnancy is associated with early life risk factors of childhood obesity, such as foetal growth-restricted infants¹⁴ and neonatal insulin resistance¹⁵, all are potential risk factors for obesity later in life. Therefore, the aim of the study was to examine the association between maternal adherence to the MD in pregnancy and the risk of overweight and abdominal obesity in 4-years old children.

METHODS

Subjects

The population-based INMA (“INfancia y Medio Ambiente”-Environment and Childhood) birth cohort study recruited 2,765 pregnant women between 2003 and 2008 in the Spanish regions of Asturias, Gipuzkoa, Sabadell and Valencia¹⁶. Inclusion criteria were: ≥ 16 years, intention to deliver at the reference hospital, ability to communicate in Spanish or regional languages, singleton pregnancy, no assisted conception. Pregnant women were recruited during prenatal visits in the first trimester of pregnancy at public health care centers or hospitals. All participants provided written informed consent, and the study was approved by hospital and institutional ethics committees in each region.

Mother-child pairs were afterwards followed at third pregnancy trimester, birth, and at child ages 6 months, 1 and 4 years. Flowchart is shown in Figure S1. The final analytic population included 1,827 pairs (66% of initially enrolled) of mothers and children.

Dietary assessment and MD

Maternal diet was assessed in the first and third trimester of pregnancy using a 101-item food-frequency questionnaire (FFQ). The FFQ was an adapted version of Willett’s questionnaire developed and validated for use among adults and pregnant women living in Spain with satisfactory coefficients for validity and reproducibility¹⁷. In the first trimester we asked mothers about their diet during the first trimester of pregnancy and in the third trimester about their diet during second and third trimesters. Standard units and serving sizes were specified

for each food item. Nutrient values and total energy intake were obtained from the US Department of Agriculture food composition tables and other published sources^{18, 19}. Adherence to the MD was assessed with the relative Mediterranean diet Score (rMED)¹³. The rMED was constructed with the average dietary data from the first and third trimester of pregnancy. The consumption of vegetables, fruits & nuts, cereals, legumes, fish, olive oil, total meat and dairy products (except alcohol because most of the pregnant women did not consumed it in our study) were measured as grams per 1,000kcal/day and were divided into tertiles. A value of 0, 1, and 2 was assigned to the intake tertiles, positively scoring higher intakes for the 6 components presumed to fit the MD. The scoring was reversed for 2 components presumed not to fit the MD (meat and dairy) positively scoring lower intakes. After summing-up scores of each component, the final score range was 0-16; It was further divided into tertiles to identify those with low, medium and high adherence to the MD.

Child Anthropometry

Repeated weight and length measures from birth to 6 months of age were extracted from medical records. For children without weight measurement available within \pm 14 days of their exact 6-month anniversary (10.2% of children), we used preexisting sex-specific growth models developed to predict weight at 6 months of age²⁰. Age- and sex-specific z-scores for weight at birth and at 6 months of age were calculated using the WHO referent²¹. Rapid growth from birth to 6 months of age was defined as a z-score weight gain greater than 0.67 SD²².

Child weight (nearest gram) and height (nearest 0.1 cm) at 4 years of age were measured by trained staff using standard protocols (without shoes and in light clothing). Child body mass

index (BMI, weight/length²) was used to estimate age- and sex-specific z-scores based on the WHO referent²¹. Overweight was defined as a BMI equal to or above the 85th percentile, and obesity as BMI z-score equal or above the 95th percentile.

At 4 years of age, waist circumference (WC) was measured at the nearest 0.1 cm using an inelastic tape (SECA model 201; SECA, Hamburg, Germany), at the midpoint between the lowest rib margin and the iliac crest, in a standing position and after a gentle expiration, by trained staff using standard protocols. This measurement was not obtained in the region of Gipuzkoa, and hence Gipuzkoa was excluded from analyses using WC. WC>90th sex-specific percentile (of the sample distribution) was considered increased risk for abdominal obesity²³. In sensitivity analysis we used waist-to-height ratio (ie, WC in cm/height in cm) and the cut-off waist-to-height ratio>0.5 as increased risk for abdominal obesity²⁴.

Covariates

Based on previous knowledge, the following variables were considered *a priori* as potential confounding factors, mediators or effect modifiers of the association between maternal diet in pregnancy and childhood obesity: study region, maternal social class and educational level, maternal pre-pregnancy BMI (based on measured height at recruitment and pre-pregnancy self-reported weight (kg/m²)), total energy intake in pregnancy, weight gain during pregnancy (extracted from prenatal visit records), maternal physical activity during pregnancy, gestational diabetes, smoking during pregnancy, maternal age at delivery, breastfeeding duration, child birth weight, child sex, rapid growth from birth to 6 months, and child age at anthropometry measurements.

Some individuals had missing values in covariables and multiple imputation was performed using chained equations, 20 completed data sets were generated and analyzed by using the standard combination rules for multiple imputations²⁵.

Statistical analysis

Descriptive statistics were used to compare socio-demographic characteristics of mother and children included in our study by BMI category of children at age 4 years. Difference in categorical variables were assessed using the X^2 test, difference in continuous variables were analysed by ANOVA.

Simple and multiple linear regression models were used to estimate the β coefficients for the association between rMED in pregnancy (expressed both in tertiles and as continuous variables per two-points increment) and BMI z-scores and WC of children at 4 years of age. Three models with different levels of adjustment were used. Only covariates which influenced the association between the exposure and outcome of interest were used for adjustment in the most adjusted model (model 3), following the backward stepwise method ($p < 0.2$). Variables that were shown to be plausible mediators of the association in mediation analyses were not included in any of the studied models (i.e. breastfeeding, for models evaluating BMI z-scores and gestational diabetes, for models evaluating WC). Model 1 was a crude model for both outcomes, Model 2 was a minimally adjusted model and Model 3 was the fully adjusted model (the covariates included for each outcome in each model can be found in tables 2 and 3).

Logistic regression models were run to study the association between rMED and the odds of offspring having overweight (including obesity) (BMI z-score \geq 85th percentile) and to have abdominal obesity (WC $>$ 90th percentile) at 4 years of age.

To assess heterogeneity among regions in the association between rMED and BMI z-score and the WC of offspring at 4 years of age, region-specific estimates were calculated by using general linear models and random-effect meta-analyses (I^2) was used to pool the estimates.

Models were also stratified by child sex, maternal pre-pregnancy BMI, smoking status, maternal physical activity, social class, educational level and infant birth weight in order to evaluate the homogeneity of effects between these subgroups of *a priori* interest. The statistical significance of interaction terms involving the exposures and these stratification variables was assessed.

As a sensitivity analysis we repeated all analyses using alternate Mediterranean Diet Score (aMED), another score of adherence of MD²⁶ developed to be applied to the US population. We compared results defining overweight using weight-for-length age- and sex-specific z-scores²¹, the IOTF criteria²⁷, and abdominal obesity using waist-to-height ratio. We also repeated BMI models excluding Gipuzkoa region from the analyses. As complementary analysis we repeated all analyses further adjusting for child diet (rMED, fibre, and fruit and vegetable intake, in different models) measured at 4 years of age. However, effects estimates did not change substantially and therefore these are not shown in the paper.

All statistical analyses were performed using the statistical package STATA 12.1 (Stata Corporation, College Station, TX).

RESULTS

We compared characteristics between mothers included in the study and those lost to follow-up; those lost to follow-up were younger and had lower social class and education level.

The characteristics of mothers and offspring according to categories of weight status of children are shown in Table 1. In our sample 16.3% of the children were overweighted and 12.4% were obese according the WHO criteria, and 14.2% overweighted and 5.7% obese according the IOTF²⁷. Mothers of obese children had higher pre-pregnancy BMI on average. Further, overweight and obese children had higher birth weight on average compared to normal weight children. The prevalence of obesity was higher in the region of Asturias (14.3%) and lower in the region of Sabadell (9.9%). Prevalence of obesity was higher in boys whereas prevalence of overweight was higher in girls.

Table 2 presents the association between maternal adherence to the MD in pregnancy, using rMED, and BMI z-score and WC at 4 years of age. The score rMED was not significantly associated with BMI z-score, in any of the studied models. There was an inverse association between the highest tertile of rMED and WC ($\beta=-0.62$; 95%CI:-1.10, -0.14; P for trend = 0.009) and also between 2 units increase of rMED and WC ($\beta=-0.18$; 95%CI:-0.33, -0.03), in models 2 and 3. We found no association between score rMED in pregnancy and odds of overweight; the observed inverse association between rMED and odds of abdominal obesity was not statistically significant (Table 3).

Stratified analyses were run by different variables of interest. No evidence of effect modification in the associations between rMED and BMI z-score were observed after stratification selected variables (Table S1).

All results were homogeneous among the four regions ($I^2=0.0\%$) (Figure S2 and S3).

DISCUSSION

In the present study, higher adherence to the MD in pregnancy was not clearly associated with BMI in 4-years old offspring, but it was modestly associated with lower waist circumference: children born of mothers with high adherence to the MD had a WC on average 0.6 cm smaller than children born to mothers with low adherence.

While this difference in WC may seem small, it is similar to that reported in previous studies evaluating adherence to the MD and WC in other populations²⁸⁻³⁰ At this early age, we do not know if this difference is clinically relevant and will have an impact on future cardiometabolic outcomes; however these results add to the evidence that early life nutritional factors might have an influence on body composition in early childhood.

To the best of our knowledge, no previous cohort study has evaluated the association between (Mediterranean) dietary patterns in pregnancy and childhood overweight and abdominal obesity risk. Previous human studies have focused on evaluating specific nutrients or foods, and results were mostly inconsistent. Maternal sugar and saturated fatty acid intakes were associated with offspring adiposity at 5 years of age⁹. Another study found no association between maternal macronutrient and energy intakes in pregnancy and offspring adiposity at 10 years of age⁷. Both maternal meat intake and protein intake were associated with adiposity in studies conducted in adolescents¹⁰. A few studies have evaluated the association between dietary patterns in pregnancy and birth outcomes, including birth weight and foetal birth restriction, potential risk factors for the obesity development later in life; however, results are also inconsistent. In a previous INMA study, diet quality measured with the Alternative Healthy Eating Index adapted for pregnancy was associated with a lower risk of delivering an infant with foetal growth restriction³¹. In another study that included INMA and a Greek birth cohort, higher adherence to the MD was associated with a lower risk of delivering a

fetal-growth-restricted infant, but only in the Spanish Mediterranean INMA regions (Sabadell and Valencia); no association was observed in the Spanish Atlantic regions (Asturias and Gipuzkoa) or the Greek cohort¹⁴. It was hypothesized that the existence of different MD patterns in the different geographic areas could explain the diverse associations observed. Degree of adherence to a Mediterranean dietary pattern in the first semester of pregnancy was associated with several features of intra-uterine growth in the Generation R birth cohort: women with low adherence had a lower placental weight and a lower birth weight³². Other studies conducted in the US, such as the Project Viva³³, did not find significant associations between diet quality measures and birth weight characteristics.

In this study, we observed no association between maternal diet in pregnancy and offspring overweight, but some evidence of an inverse association between diet quality in pregnancy and offspring waist circumference, a marker of abdominal obesity. As pointed out in different studies, maternal weight status seems to be the strongest predictor of obesity later in life⁴. Some animal experiments have argued that maternal obesity – as a consequence of a high-fat diet – but not a high-fat diet *per se*, is necessary to program obesity predisposition in the offspring^{5,6}. Also, studies in animals have observed that post-natal offspring diet seems to combine with prenatal maternal diet in exacerbating obesity risk⁵. In rodents the consumption of a western diet during lactation is particularly critical for the ability of a maternal western diet to cause obesity and associated metabolic consequences in childhood⁵. Also, it has been speculated that the effects of intrauterine over-nutrition are hard to detect early in childhood and may predominantly appear later in life. This hypothesis is supported by studies of offspring exposed to gestational diabetes, where it seems that this effect on childhood obesity becomes apparent from the age of 9-10 years but not earlier³⁴. Finally, it may be possible that MD in pregnancy has a specific effect on programming body fat

distribution leading to a lower abdominal obesity risk without influencing general obesity. As observed in this cohort, children born of mothers with higher adherence to MD in pregnancy tend to show a lower WC. This is consistent with previous studies that shown that MD influence abdominal obesity independently of total body weight in adults and children²⁸.

This study has several limitations: diet was evaluated in pregnancy (both first and third trimester), but not during lactation, which might be a critical window of exposure to program later obesity risk. Nevertheless, results were similar using both first and third trimester dietary data, and it is unlikely that dietary patterns change drastically in lactation compared to third trimester³⁵. Diet was evaluated using a food frequency questionnaire, subjected to measurement errors that may led to an attenuation of effect estimates. Energy adjusted dietary data was used in order to try to decrease measurement error. Overweight in children was evaluated using anthropometrical measurements at 4 years of age. It may be too early to detect any potential effect of diet on childhood obesity risk and hence further studies are needed at older ages. Also, other indicators of visceral fat accumulation, such as precise measurements of body composition would be necessary to confirm these findings. Finally, as in any observational study, residual confounding and selection bias cannot totally be ruled out. In this sense, there may be other postpartum unmeasured variables associated with the adherence to MD that may contribute to abdominal adiposity.

The strengths of the present study include the use of a large population-based birth cohort study set up in several geographical areas of Spain and the prospective design. We used a validated FFQ¹⁷, and anthropometric indicators were measured and not self-reported. We assessed several potential confounders and we also evaluated the potential

mediating effect of these mentioned variables. We also conducted several sensibility and effect modification analyses to test the robustness of our findings.

In conclusion, our data suggests that MD during pregnancy is not associated with measures of overweight in 4 years old offspring, but is inversely associated with offspring waist circumference, a marker of abdominal obesity. Long term studies with a larger sample size, better measurements of body fat distribution, and biomarkers of cardio-metabolic risk are needed to disentangle the plausible effect of MD in pregnancy on visceral fat accumulation in childhood.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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A full roster of the INMA Project Investigators can be found at

http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html

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The author's responsibilities were as follows: DR designed the research; SFB and DM analyzed data; SFB and DR wrote the manuscript, with close assistance from DV, JV, EMNM, and MV, taking into account comments and suggestions of other co-authors; JS

is the overall coordinator of the INMA project which was conceptualized, designed, and implemented in collaboration with the principal investigators in the collaborating centers. SFB, DR had primary responsibility for the final content; all authors read and approved the final manuscript.

REFERENCES

1. de Onis M, Blössner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. *Am J Clin Nutr* 2010; **92**: 1257-64.
2. Durmus B, Arends LR, Ay L, *et al.* Parental anthropometrics, early growth and the risk of overweight in pre-school children: the Generation R Study. *Pediatr Obes* 2013; **8**: 339-50
3. Henriksson P, Eriksson B, Forsum E, Lof M. Gestational weight gain according to Institute of Medicine recommendations in relation to infant size and body composition. *Pediatr Obes* 2015; **10**: 388-94.
4. Rooney BL, Mathiason MA, Schauberger CW. Predictors of obesity in childhood, adolescence, and adulthood in a birth cohort. *Matern Child Health J* 2011; **15**: 1166-75.
5. Parlee SD, MacDougald OA. Maternal nutrition and risk of obesity in offspring: the Trojan horse of developmental plasticity. *Biochimica et biophysica acta* 2014; **1842**: 495-506.
6. White CL, Purpera MN, Morrison CD. Maternal obesity is necessary for programming effect of high-fat diet on offspring. *Am J Physiol Regul Integr Comp Physiol* 2009; **296**: R1464-72.

7. Brion MJ, Ness AR, Rogers I, *et al.* Maternal macronutrient and energy intakes in pregnancy and offspring intake at 10 y: exploring parental comparisons and prenatal effects. *Am J Clin Nutr* 2010; **91**: 748-56.
8. Donnelly JM, Walsh JM, Byrne J, Molloy EJ, McAuliffe FM. Impact of maternal diet on neonatal anthropometry: a randomized controlled trial. *Pediatr Obes* 2015; **10**: 52-6.
9. Murrin C, Shrivastava A, Kelleher CC, Lifeways Cross-generation Cohort Study Steering G. Maternal macronutrient intake during pregnancy and 5 years postpartum and associations with child weight status aged five. *Eur J Clin Nutr* 2013; **67**: 670-9.
10. Maslova E, Rytter D, Bech BH, *et al.* Maternal protein intake during pregnancy and offspring overweight 20 y later. *Am J Clin Nutr* 2014; **100**: 1139-48.
11. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002; **13**: 3-9.
12. Tognon G, Hebestreit A, Lanfer A, *et al.* Mediterranean diet, overweight and body composition in children from eight European countries: cross-sectional and prospective results from the IDEFICS study. *Nutr Metab Cardiovasc* 2014; **24**: 205-13.

13. Romaguera D, Norat T, Vergnaud AC, *et al.* Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. *Am J Clin Nutr* 2010; **92**: 912-21.
14. Chatzi L, Mendez M, Garcia R, *et al.* Mediterranean diet adherence during pregnancy and fetal growth: INMA (Spain) and RHEA (Greece) mother-child cohort studies. *Br J Nutr* 2012; **107**: 135-45.
15. Gesteiro E, Rodriguez Bernal B, Bastida S, Sanchez-Muniz FJ. Maternal diets with low healthy eating index or Mediterranean diet adherence scores are associated with high cord-blood insulin levels and insulin resistance markers at birth. *Eur J Clin Nutr* 2012; **66**: 1008-15.
16. Guxens M, Ballester F, Espada M, *et al.* Cohort Profile: the INMA--Infancia y Medio Ambiente--(Environment and Childhood) Project. *Int J Epidemiol* 2012; **41**: 930-40.
17. Vioque J, Navarrete-Munoz EM, Gimenez-Monzo D, *et al.* Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. *Nutr J* 2013; **12**: 26.
18. US Department of Agriculture ARS. USDA National Nutrient Database for Standard Reference, Release 21. In, 2008.

19. Palma I FA, Cantós D. *Tablas de Composición de Alimentos por Medidas Caseras de Consumo Habitual en España*. CESNID, McGraw-Hill Interamerican: Madrid, 2008.
20. Valvi D, Casas M, Mendez MA, *et al*. Prenatal bisphenol a urine concentrations and early rapid growth and overweight risk in the offspring. *Epidemiology* 2013; **24**: 791-9.
21. WHO Child Growth Standards: *Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development*. In. Geneva: World Health Organization 2006. Available at: <http://www.who.int/childgrowth/standards/en/>
(<http://www.who.int/childgrowth/standards/en/>).
22. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ* 2000; **320**: 967-71.
23. Fernandez JR, Redden DT, Pietrobelli A, Allison DB. Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. *J Pediatr* 2004; **145**: 439-44.

24. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0.5 could be a suitable global boundary value. *Nutr Res Rev* 2010; **23**: 247-69.
25. Spratt M, Carpenter J, Sterne JA, *et al.* Strategies for multiple imputation in longitudinal studies. *Am J Epidemiol* 2010; **172**: 478-87.
26. Fung TT, Rexrode KM, Mantzoros CS, Manson JE, Willett WC, Hu FB. Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation* 2009; **119**: 1093-100.
27. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000; **320**: 1240-3.
28. Romaguera D, Norat T, Mouw T, *et al.* Adherence to the Mediterranean diet is associated with lower abdominal adiposity in European men and women. *J Nutr* 2009; **139**: 1728-37.
29. Funtikova AN, Benitez-Arciniega AA, Gomez SF, Fito M, Elosua R, Schroder H. Mediterranean diet impact on changes in abdominal fat and 10-year incidence of abdominal obesity in a Spanish population. *Br J Nutr* 2014; **111**: 1481-7.

30. Schroder H, Mendez MA, Ribas-Barba L, Covas MI, Serra-Majem L. Mediterranean diet and waist circumference in a representative national sample of young Spaniards. *Int J Pediatr Obes* 2010; **5**: 516-9.
31. Rodriguez-Bernal CL, Rebagliato M, Iniguez C, *et al.* Diet quality in early pregnancy and its effects on fetal growth outcomes: the Infancia y Medio Ambiente (Childhood and Environment) Mother and Child Cohort Study in Spain. *Am J Clin Nutr* 2010; **91**: 1659-66.
32. Timmermans S, Steegers-Theunissen RP, Vujkovic M, *et al.* The Mediterranean diet and fetal size parameters: the Generation R Study. *Br J Nutr* 2012; **108**: 1399-409.
33. Rifas-Shiman SL, Rich-Edwards JW, Kleinman KP, Oken E, Gillman MW. Dietary quality during pregnancy varies by maternal characteristics in Project Viva: a US cohort. *J Am Diet Assoc* 2009; **109**: 1004-11.
34. Tanvig M. Offspring body size and metabolic profile - effects of lifestyle intervention in obese pregnant women. *Dan Med J* 2014; **61**: B4893.
35. Sotres-Alvarez D, Herring AH, Siega-Riz AM. Latent transition models to study women's changing of dietary patterns from pregnancy to 1 year postpartum. *Am J Epidemiol* 2013; **177**: 852-61.

Table 1: Descriptive characteristics of mothers and children, by weight status of children at 4 years of age.

Table 2: Association between maternal relative Mediterranean diet score in pregnancy and Body Mass Index (BMI, z-score) and Waist Circumference (WC, cm) at 4 years of age.

Table 3: Association between maternal relative Mediterranean diet score in pregnancy and odds of having overweight and abdominal obesity at 4 years of age

Table 1 Characteristics of mothers and children, by weight status of children at 4 years of age

	n= 1827	Normal weight (n=1303, 71.3%)	Overweight (n=298, 16.3 %)	Obese (n=226, 12.4 %)	P
Child characteristics					
Sex (%)					
Male	941	73.5	12.8	13.7	0.000
Female	886	69.0	20.1	10.9	
Age years (mean (s.d.))	1827	4.4 (0.2)	4.4 (0.2)	4.4 (0.2)	0.268
Region (%)					
Asturias	385	68.5	17.3	14.2	0.002
Gipuzkoa	395	64.6	21.8	13.6	
Sabadell	474	77.1	13.0	9.9	
Valencia	563	73.1	14.5	12.4	
Birth weight (mean (s.d.))	1794	3307.0 (403.2)	3423.4 (392.2)	3450.0 (362.5)	0.000
Breastfeeding duration (%)					
0 weeks	259	73.7	14.7	11.6	0.686
>0-16 weeks	437	74.1	14.4	11.5	
>16-24 weeks	286	71.0	16.8	12.2	
>24 weeks	812	69.4	17.4	13.2	
Maternal characteristics					
Age at delivery (mean (s.d.))	1827	31.0 (4.1)	30.7 (4.1)	31.1 (4.0)	0.567
Smoking in pregnancy (%)					
No cig/d	1493	71.9	16.2	11.9	0.233
1-4 cig/d	110	62.7	17.3	20.0	
>4-7 cig/d	93	68.8	19.3	11.8	
>7 cig/d	100	74.0	13.0	13.0	
Social class (%)					
I+II	441	73.9	16.1	10.0	0.289
III	507	72.4	15.8	11.8	
IV+V	878	69.4	16.7	13.9	
Education level (%)					
Primary or less	383	69.2	17.8	13.0	0.376
Secondary	762	71.0	15.4	13.6	
University	678	72.7	16.7	10.6	
Physical activity in pregnancy(%)					
Sedentary	131	71.8	13.7	14.5	0.213
Little active	464	70.3	15.3	14.4	
Moderately active	736	70.4	16.6	13.0	
Quite-Very active	478	73.8	17.2	9.0	
Pre-pregnancy BMI (mean (s.d.))	1827	23.1 (3.9)	24.1 (4.3)	25.9 (5.0)	0.000
Pregnancy weight gain, kg (mean (s.d.))	1776	13.7 (4.9)	13.6 (5.0)	14.4 (6.0)	0.119
Pregnancy EI, kcal (mean (s.d.))	1827	2074.4 (463.4)	2067.4 (431.3)	2024.8 (470.7)	0.325
Diabetes (%)					
None	1340	70.6	16.7	12.7	0.691
Impaired glucose tolerance	160	72.5	16.9	10.6	
Gestational DM	78	65.4	15.4	19.2	
DM before pregnancy	5	60.0	20.0	20.0	
rMED (mean(s.d.))	1827	8.0 (2.6)	8.2 (2.6)	8.0 (2.4)	0.504

Variables defined as: overweight: BMI >85th percentile - ≤95th percentile; obese: BMI >95th percentile of WHO reference.

Abbreviations: BMI: body mass index; EI: Energy Intake; DM: diabetes mellitus. rMED: relative Mediterranean Diet Score.

Table 2 Association between maternal relative Mediterranean diet score in pregnancy and Body Mass Index (BMI, z-score) and Waist Circumference (WC, cm) at 4 years of age.

rMED		T ₁	T ₂	T ₃		2 units increase
	Range:	(1-7)	(8-9)	(10-15)		
			β (95% CI)	β (95% CI)	<i>P for trend</i>	β (95% CI)
BMI¹						
(n=1827)						
	Model 1	Ref	0.00 (-0.11, 0.12)	-0.01 (-0.13, 0.10)	0.827	0.01 (-0.03, 0.04)
	Model 2	Ref	-0.02 (-0.13, 0.10)	-0.07 (-0.20, 0.05)	0.255	-0.01 (-0.05, 0.03)
	Model 3	Ref	-0.06 (-0.17, 0.05)	-0.09 (-0.20, 0.02)	0.113	-0.02 (-0.06, 0.01)
WC²						
(n=1398)						
	Model 1	Ref	0.13 (-0.42, 0.69)	-0.05 (-0.53, -0.64)	0.806	0.12 (-0.07, 0.30)
	Model 2	Ref	-0.26(-0.73, 0.20)	-0.57 (-1.07, -0.07)	0.024	-0.15 (-0.31, 0.00)
	Model 3	Ref	-0.34 (-0.78, 0.11)	-0.62(-1.10, -0.14)	0.009	-0.18 (-0.33, -0.03)

rMED: relative Mediterranean Diet Score: range from 1-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3)

adherence to the Mediterranean diet.

¹Model 1: Crude model. General linear regressions with no adjustments.

Model 2: General linear regressions adjusted for child sex, region, child age, maternal total energy intake.

Model 3: General linear regressions adjusted for child sex, region, child age, maternal total energy intake, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months.

²Model 1: Crude model General linear regressions with no adjustments.

Model 2: General linear regressions adjusted for child sex, region, child age, maternal total energy intake and child height.

Model 3: General linear regressions adjusted for child sex, region, child age, maternal total energy intake, child height, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight, rapid growth from birth to 6 months and breastfeeding duration.

BMI: body mass index; CI: Confidence Interval; rMED: relative Mediterranean Diet Score; Waist Circumference

Table 3 Association between maternal relative Mediterranean diet score in pregnancy and odds of having overweight and abdominal obesity at 4 years of age

rMED	Range:	T ₁ (1-7)	T ₂ (8-9) OR (CI)	T ₃ (10-15) OR (CI)	<i>P for trend</i>	2 units increase OR (CI)
Overweight¹						
(n=1827)	Model 1	Ref	1.06 (0.83, 1.35)	1.08 (0.85, 1.39)	0.506	1.04 (0.96, 1.12)
	Model 2	Ref	0.99 (0.78, 1.28)	0.97 (0.75, 1.26)	0.833	1.00 (0.92, 1.09)
	Model 3	Ref	0.88 (0.67, 1.15)	0.94 (0.71, 1.24)	0.596	0.98 (0.89, 1.07)
Abdominal obesity²						
(n=1398)	Model 1	Ref	0.95 (0.63, 1.41)	0.82 (0.53, 1.27)	0.378	1.00 (0.87, 1.15)
	Model 2	Ref	0.90 (0.58, 1.39)	0.62 (0.38, 1.02)	0.068	0.89 (0.48, 1.04)
	Model 3	Ref	0.84 (0.53, 1.32)	0.62 (0.37, 1.03)	0.064	0.89 (0.76, 1.05)

rMED: relative Mediterranean Diet Score: range from 1-15, in 3 tertiles, to define: low (T1), medium (T2) and high (T3) adherence to the Mediterranean diet.

Variables defined as: general obesity: BMI >85th percentile of WHO reference; abdominal obesity: Waist Circumference > 90th percentile distribution of the sample.

¹Model 1: Crude model. Logistic regressions with no adjustments.

Model 2: Logistic regressions adjusted for child sex, region, child age, maternal total energy intake.

Model 3: Logistic regressions adjusted for child sex, region, child age, maternal total energy intake, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, gestational diabetes, child birth weight and rapid growth from birth to 6 months.

²Model 1: Crude model. Logistic regressions with no adjustments.

Model 2: Logistic regressions adjusted for child sex, region, child age, maternal total energy intake and child height.

Model 3: Logistic regressions adjusted for child sex, region, child age, maternal total energy intake, child height, educational level, smoking status, maternal physical activity, maternal pre-pregnancy BMI, weight gain during pregnancy, child birth weight, rapid growth from birth to 6 months and breastfeeding duration.

CI: Confidence Interval; OR: odds ratio; rMED: relative Mediterranean Diet Score.