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Effects of Mediterranean diet during pregnancy on the onset of overweight or obesity in the offspring: a randomized trial

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BACKGROUND/OBJECTIVES: The PREMEDI study was designed to assess the efficacy of nutritional counseling aimed at promoting Mediterranean Diet (MD) during pregnancy on the incidence of overweight or obesity at 24 months in the offspring.
METHODS: PREMEDI was a parallel-arm randomized-controlled trial. 104 women in their first trimester of pregnancy were randomly assigned in a 1:1 ratio to standard obstetrical and gynecological care alone (CT) or with nutritional counseling promoting MD. Women enrolled in the MD arm were provided with 3 sessions of nutritional counseling (one session per trimester). The main outcome was the proportion of overweight or obesity among the offspring at the age of 24 months. Maternal MD-adherence and weight gain during pregnancy were also evaluated. Lastly, the evaluation of epigenetic modulation of metabolic pathways in the offspring was analyzed in cord blood.
RESULTS: Five women in the MD arm and 2 in the CT arm were lost to follow-up, so a total of 97 completed the study. At 24 months, children of MD mothers were less likely to have overweight or obesity than those of the CT mothers (6% vs. 30%, absolute risk difference = −24%, 95% CI −38% to −9%, $p = 0.003$, number needed to treat 4, 95% CI 2 to 12, per-protocol analysis). A significantly higher increase of MD-adherence during the trial was observed in the MD arm compared to the CT arm. A similar body weight gain at the end of pregnancy was observed in the two arms. The mean (SD) methylation rate of the leptin gene in cord blood was 30.4 (1.02) % and 16.9 (2.99) % in the MD and CT mothers, respectively ($p < 0.0001$).
CONCLUSIONS: MD during pregnancy could be an effective strategy for preventing pediatric overweight or obesity at 24 months. This effect involves, at least in part, an epigenetic modification of leptin expression.

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INTRODUCTION

The current epidemic of pediatric obesity is a major public health issue that calls for preventive strategies to limit the disease burden [1]. The initial stages of life provide a window of opportunity to prevent the occurrence of overweight or obesity and attain long-term health outcomes [2]. The future disease susceptibility can be influenced by nutritional exposures during this critical period of life [3]. Maternal diet during pregnancy has been linked to obesity in the offspring and could be a potential target for disease prevention [4]. The Mediterranean Diet (MD) is one of the healthiest dietary patterns, providing considerable amounts of fiber, antioxidants, polyphenols, vitamins, and a balanced ratio of essential fatty acids. This has a beneficial impact on overall health and prevents the occurrence of excess weight in adults [5]. During pregnancy, MD may protect against overweight or obesity in the offspring, possibly influencing fetal programming by epigenetic modulation of gene expression [6–8]. Currently, the evidence regarding the effect of MD during pregnancy on the prevention of childhood overweight/obesity is limited and based on observational studies [9–11]. The Mediterranean Diet during Pregnancy (PREMEDI) trial was a randomized controlled trial (RCT) aimed at addressing such limitation.

METHODS

Trial design

PREMEDI was a randomized, parallel-group, controlled trial aimed at evaluating the effects of MD during pregnancy on the prevention of overweight/obesity at 24 months in the offspring.

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Participants

All pregnant women in their first trimester of pregnancy consecutively observed at a Tertiary Center for Gynecology and Obstetrics were evaluated for the study. These subjects were evaluated by a Research Team (RT) composed of gynecologists and dietitians experienced in clinical trials. The RT evaluated the presence of inclusion and exclusion criteria, provided information regarding study design and outcomes, and collected written informed consent from the women.

Inclusion criteria were Caucasian ethnicity and age range of 20 to 35 years. Exclusion criteria were documented infections during pregnancy, twin pregnancy, malignancies, malformations, immunodeficiency, diabetes mellitus and other chronic diseases; chronic inflammatory bowel diseases; gastrointestinal functional disorders; celiac disease; a history of abdominal surgery; neurological and neuropsychiatric disorders; and the utilization of a vegan diet.

Intervention

Soon after the collection of the written informed consent, the study subjects were randomly allocated in a 1:1 ratio to the CT or MD arms by the RT.

Women enrolled in the CT group were provided standard-of-care recommendations by the gynecologists operating at the Center. Such recommendations included energy intake, physical activity, optimal weight gain during pregnancy based on pre-pregnancy weight [12], and hygiene rules for food-related illnesses [13]. Whereas, women enrolled in the MD group, received standard obstetrical and gynecological care plus personalized MD nutritional counseling provided by certified dietitians operating at the Center. The MD nutritional counseling was performed in three face-to-face sessions at enrollment (8–13 gestational weeks), 3 months (14–28 gestational weeks) and 6 months after the enrollment (29–40 gestational weeks). MD nutritional counseling was based on the following recommendations: use of extra virgin olive oil as the main cooking fat (at least 4 tablespoons/day); intake of 2 servings/day of vegetables; intake of 3 servings/day of fruit, avoiding juices; intake of 3 servings/day of wholegrain cereals; intake of 3 servings/day of skimmed dairy products; intake of 3 servings/week of legumes; intake of 3 servings/week of fish; intake of 3 servings/week of nuts and seeds; intake of 2 liters/day of water; low consumption of red meat and processed meat; and avoidance of refined grains and ultraprocessed foods including processed baked goods, pre-sliced bread, soft drinks, fruit juices, and precooked meals [14].

Outcomes

The primary outcome was the proportion of children with overweight or obesity at 24 months of age in the MD vs. CT arm, as detected by the International Obesity Task Force (IOTF) growth charts [15]. The analysis of the primary outcome was prespecified as intention to treat (ITT). Other outcomes were maternal adherence to MD, as detected by the MedDiet Score [16], maternal weight gain, and epigenetic modulation of metabolic pathways in the offspring. These non-primary outcomes were analyzed using per-protocol analysis (PPA).

Sample size calculation

Sample size was calculated based on the primary outcome, i.e., the proportion of offspring with overweight or obesity at 24 months. Based on prior observational studies, we expected an incidence of overweight or obesity at 24 months of 25%. To detect an absolute difference of 20% in the proportion of overweight or obesity at 24 months at an alpha level of 0.05 and with a power of 80%, 49 mother-child pairs per group were necessary (Pearson Chi-square test). Assuming a dropout rate of up to 5% as in our previous studies, we enrolled 52 mother-child pairs per group, for a total of 104 pairs.

Randomization

A central randomization procedure was applied to allocate women in a 1:1 ratio into the MD and CT arms. The randomization list was generated by applying the *ralloc* command with block sizes of 2 in Stata version 14.2 (Stata Corporation, College Station, TX, USA) [17].

Allocation concealment

The treatments were consecutively numbered according to the randomization list, which was known only to the study coordinator [18, 19].

Blinding

Blinding the women was not possible because of the nature of the intervention. The collection of main study outcome data was performed by physicians and pediatric nurses unaware of the study outcome and group assignment.

Study monitoring and data management

Study monitoring was performed by an independent clinical trial monitor and included on-site visits and telephone interviews with the investigators. The monitor reviewed the study forms for completeness, clarity, and consistency and instructed the researchers to make any needed corrections or additions. The clinical researchers entered data in a case report form. Such data was anonymized and entered into an electronic database by the same researcher. The database underwent data cleaning according to standard procedures and was locked before statistical analysis, performed by a statistician.

Data collection

At enrollment, a multidisciplinary team composed of gynecologists and certified dietitians operating at the Center evaluated the following variables: anamnestic, clinical, and anthropometric features of all women enrolled in the study, socio-demographic factors, gestational age, allergies, number of cohabitants, pets, physical activities, use of drugs, smoking exposure, education level, family and living conditions. The MedDiet Score, a validated 14-item questionnaire assessing MD adherence, was administered by the dietitians involved in the multidisciplinary team [16]. Each item has a score of 0 or 1, with a total score ranging from 0 to 14. Adequate adherence to MD was determined as a MedDiet score ≥9. All data were collected anonymously in a dedicated clinical chart. The intake of dietary supplements, pre-, pro-, and symbiotics were recorded in the same chart. Follow-up visits were scheduled at gestational weeks 8 to 13, 14 to 28, and 29 to 40. During these visits, the multidisciplinary team performed a full anamnestic and physical examination and assessed the MedDiet Score. A cord blood sample (≥10 ml) was collected by the gynecologists at delivery in the first 11 women enrolled in each arm. For all babies after delivery, a follow-up visit was planned every 3 months for the first 12 months of life and then every 6 months until the age of 2 years. At each visit, a team composed of pediatricians, allergists and pediatric nurses, unaware of study aims and group assignment, collected data regarding anamnestic and clinical features, body growth, occurrence of allergic diseases and/or other chronic disease, and antibiotic use. The diagnosis of overweight or obesity in the offspring at 24 months of age was made using the International Obesity Task Force (IOTF) body mass index (BMI) cut-offs [15]. Anthropometric measurements (weight and length) were collected following standardized procedures. Briefly, subjects were weighed naked twice on calibrated electronic scales (Seca 834) or on mechanical scale (Seca 711). Supine length of infants was measured twice using a standard measuring board (Seca 210 Mobile Measuring mat). If the anthropometric measures deviated substantially (>100 g for weight and >5 mm for length), a third measurement was obtained.

DNA isolation from cord blood, methylome analyses and ultra-deep DNA methylation at leptin gene

Cord blood samples were collected at the time of delivery in EDTA tubes. Genomic DNA was extracted using the DNA Extraction Kit (GE Healthcare, Uppsala, Sweden) following the manufacturer's protocol. Methylome analyses were performed by using Epic Array Illumina 850k. Bioinformatic analyses were performed on IDAT files by applying RnBeads R-based scripts [20, 21]. As a first step, the quality score was determined. According to sample annotations, batch effects and phenotype covariates were identified. DNA methylation distributions and intergroup as well as intragroup variability in methylation profiles were analyzed. Differential methylation between groups of samples was calculated. Differentially methylated CpG sites, promoters and CpG island were calculated among single samples and between groups using the Mann-Whitney U test. According to the dissimilarities in terms of DNA methylation at each of the 850k CpG sites, a Principal Component Analysis (PCA) was performed, and PCA plots were generated. To analyze DNA methylation at the Leptin gene, we generated an amplicon library for sequencing as previously described [22, 23]. Briefly, genomic DNA was submitted to bisulfite treatment and a double amplification strategy was adopted. The first PCR step was performed using bisulfite-specific Leptin primers with Hot Start Taq (Qiagen) and with the following temperature conditions: 95 °C for 15 min;

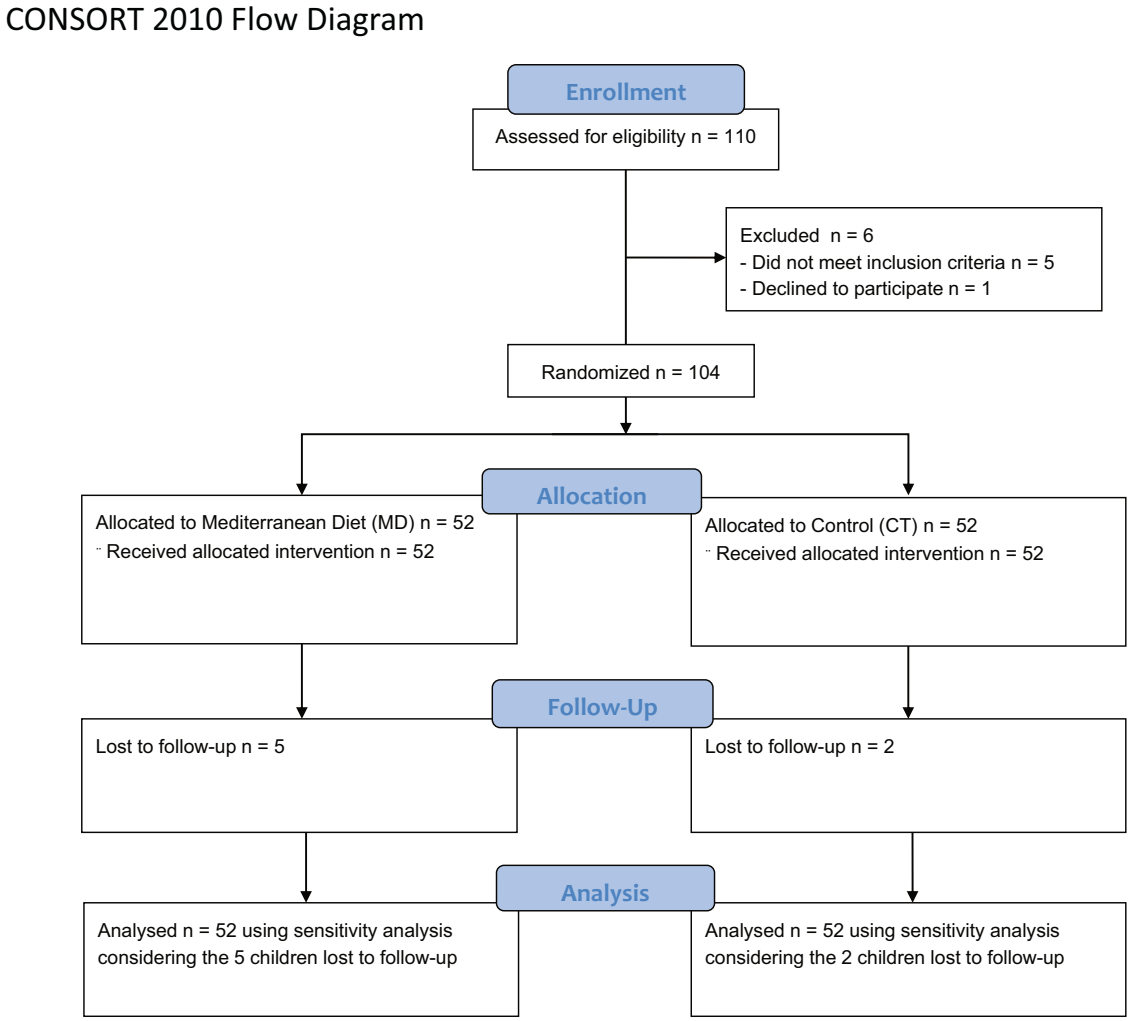

Fig. 1 CONSORT 2010 Flow Diagram. Flow diagram of the PREMEDI randomized controlled trial.

36 cycles of denaturation at 95 °C for 30 s; annealing at 53 °C for 40 s; and elongation at 72 °C for 1 min and 72 °C for 10 min. The second PCR protocol was performed to add multiplexing indices to the first amplicons (forward and reverse "Nextera XT" primers, Illumina, San Diego, CA, USA). The Master Mix KAPA Uracil plus (Roche, Basel, Switzerland) was used for the second amplification and the PCRs were performed with the following temperature conditions: 95 °C for 3 min; 12 cycles of denaturation at 98 °C for 20 s; annealing at 55 °C for 30 s: and elongation at 72 °C for 50 s and 72 °C for 5 min. Both PCR steps were followed by purification using magnetic Beads (Beckman-Coulter, Brea, CA, USA) according to the manufacturer's instructions. All amplicons were quantified using a Qubit 2.0 fluorometer. An equimolar amplicon library was generated and then diluted to a final concentration of 8 pM. Phix control library (Illumina) [10% (v/v)] was added to increase diversity of base calling during sequencing. The library was subjected to sequencing using V2-nano reagent kits on the Illumina MiSeq system (Illumina).

Statistical analysis

Continuous variables were reported as median (50th percentile) and interquartile interval (IQI, 25th and 75th percentiles). Discrete variables were reported as the number and proportion of subjects with the characteristic of interest. The main outcome, i.e., the proportion of children with overweight or obesity at 24 months, was analyzed using the prespecified Pearson's Chi-square test (see sample size calculation). We also calculated the risk difference and its 95% confidence using a binomial regression model (BRM) having the proportion of children with overweight or obesity as the response variable, and treatment (discrete: 0 = CT; 1 = MD) as the predictor variable (ITT and PPA). We evaluated the influence of baseline maternal weight or BMI on the risk difference by adding them as covariables to the BRM (PPA). Other outcomes, i.e., maternal adherence to treatment and weight gain during pregnancy were analyzed using random effect linear regression (RELR) models (PPA). To this aim, MedDiet Score (continuous, score) or maternal body weight (continuous, kg) was the response variable, treatment (discrete: 0 = CT; 1 = MD); trimester (discrete: 0 = 1st trimester; 1 = 2nd trimester; 2 = 3rd trimester), a treatmentXtime (discreteXdiscrete) interaction were the predictors (PPA), and the mother was the random effect [24]. We evaluated the influence of baseline maternal weight or baseline maternal BMI on weight gain by adding it as covariable to the RELR model (PPA). Three Bonferroni corrected between-group (MD vs. CT) within-time (1st, 2nd and 3rd trimester) contrasts were calculated. The between-group differences in the methylation of the leptin gene were analyzed using unpaired Student's *t*-test. Statistical analysis was performed using Stata 18.5 (Stata Corporation, College Station, TX, USA) and GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Figure 1 reports the flow of women through the study. A total of 110 women were assessed for eligibility, 5 subjects were excluded because the presence of exclusion criteria, and 1 woman refused to participate. The remaining 104 women were randomized in a 1:1 ratio into the MD or CT arm. 5 of 52 (9.6%) women in the MD arm and 2 of 52 (3.8%) women in the CT arm were lost to follow-up.

Table 1 shows the main baseline features of the women evaluated into the study. The two study groups presented similar baseline demographic, anamnestic and clinical features.

The gestational age was similar in the two study groups. The mean (95%CI) gestational age was 39 (38 to 39) weeks in the CT arm and 39 (39 to 40) weeks in the MD arm (PPA, $p = 0.140$, RELR).

A slight, although significantly higher mean body weight at birth was observed in the offspring from MD if compared to CT group (3391, 3287 to 3495 g in the MD group vs. 3189, 3089 to 3290 g in the CT, $p = 0.006$, RELR).

Table 1. Baseline features of the women enrolled in the PREMEDI randomized controlled trial.

	MD arm	CT arm
N	52	52
Age (years)	31 (28–34)	32 (29–34)
Pre-pregnancy weight (kg)	63.0 (56.0–69.0)	60.0 (54.0–72.0)
Pre-pregnancy height (m)	1.65 (1.62–1.68)	1.62 (1.60–1.68)
Pre-pregnancy BMI (kg/m^2)	23.6 (20.6–25.4)	23.0 (20.0–27.0)
Pre-pregnancy BMI-NIH		
Underweight	5 (9.6%)	6 (11.5%)
Normal weight	32 (61.5%)	27 (51.9%)
Overweight	12 (23.1%)	16 (30.8%)
Obesity	3 (5.8%)	3 (5.8%)
Number of cohabitants	2 (1–2)	2 (2–3)
Has allergy	25 (28.8%)	16 (30.7%)
Has pets	12 (23.1%)	12 (23.1%)
Has regular physical activity	4 (7.7%)	3 (5.8%)
Smokes cigarettes	7 (13.5%)	7 (13.5%)
Education		
Middle school	4 (7.7%)	9 (17.3%)
High school	27 (51.9%)	27 (51.9%)
University	21 (40.4%)	16 (30.8%)
MedDiet score	7.0 (5.2–9.0)	6.5 (5–7.7)

Continuous variables are reported as median (50th percentile) and interquartile interval (IQI, 25th and 75th percentiles). Discrete variables are reported as the number and proportion of subjects with the characteristic of interest.
MD Mediterranean diet arm, *CT* control arm, *BMI* body mass index, *NIH* National Institutes of Health.

Results of the main study outcome: incidence of overweight or obesity at 24 months

Table 2 reports the incidence of overweight or obesity in the MD and CT arms.

At PPA, the absolute risk difference of overweight or obesity for MD vs. CT was −0.24 (95% CI −0.38 to −0.09), corresponding to a number needed to treat (NNT) of 4 (95% CI 2 to 12). Such risk difference was virtually unmodified when baseline maternal weight (−0.24, 95% CI −0.38 to −0.09) or baseline maternal BMI (−0.23, 95% CI −0.38 to −0.09) were entered into a bivariable BRM.

At ITT, the absolute risk difference was maintained under the best-case scenario, with MD children lost to follow-up ($n = 5$) assigned a positive outcome and with CT children lost to follow-up ($n = 2$) assigned a negative outcome (−27%, 95% CI −41% to −13%), but under the worst-case scenario, i.e., with MD children lost to follow-up ($n = 5$) assigned a negative outcome and with CT children lost to follow-up ($n = 2$) assigned a positive outcome, declined below the frequency of 20% that we could detect with the available sample size, even if the sign of the absolute risk difference was preserved (−14%) and its 95%CI (−29% to 2%) were still compatible with a mostly positive effect of reduction of overweight and obesity.

Results of the other study outcomes

Figure 2 plots the changes of the MedDiet score in the MD and CT arms at the 1st, 2nd, and 3rd trimester of pregnancy (PPA). There was an increase in the MedDiet Score during the trial in the MD arm compared to the CT arm. The mean (Bonferroni corrected 95% CI) difference of the MedDiet score in the MD group vs. CT group was 0.7 (−0.1 to 1.6, Bonferroni corrected p value = 0.08) at the 1st trimester, 3.0 (2.2 to 3.8, Bonferroni corrected p value < 0.0001) at the 2nd trimester and 4.1 (3.2 to 4.9, Bonferroni corrected p value < 0.0001) at the 3rd trimester of pregnancy. The mean (95% CI) of the MedDiet score in the MD arm was ≥9, meaning excellent MD-adherence, already starting from the 2nd trimester.

As estimated by RELR using baseline weight as covariate (PPA), the mean (95% CI) weight was 67.3 (66.4 to 68.1) kg in the CT arm and 67.2 (66.4 to 68.1) kg in the MD arm at the 1st trimester; 70.6 (69.8 to 71.4) kg in the CT arm and 71.2 (70.3 to 72.0) kg in the MD arm at the 2nd trimester; and 75.9 (75.1 to 76.8) kg in the CT arm and 75.7 (74.9 to 76.6) kg in the MD arm at the 3rd trimester. The corresponding weight change for the MD vs. the CT arm was −0.01 (Bonferroni-corrected 95% CI −1.5 to 1.4) kg at the 1st trimester, 0.6 (−0.8 to 2.0) at the 2nd trimester and −0.2 (−1.7 to 1.2) at the 3rd trimester of pregnancy (Bonferroni-corrected p value ≥ 0.95 for all comparisons). Thus, maternal weight gain was similar in the CT and MD arm and was not affected by baseline

Table 2. Incidence of overweight or obesity at 24 months in the offspring.

	Per-protocol analysis	Intention to treat analysis-best-case scenario*	Intention to treat analysis-worst-case scenario**
MD event rate			
n/N	3/47	3/52	8/52
Proportion (95%CI)	0.06 (0.01 to 0.17†)	0.06 (0.01 to 0.16†)	0.15 (0.07 to 0.28†)
CT event rate			
n/N	15/50	17/52	15/52
Proportion (95%CI)	0.30 (0.18 to 0.45†)	0.33 (0.20 to 0.47†)	0.29 (0.17 to 0.43†)
Absolute risk difference (MD-CT)	−0.24 (−0.38 to −0.09††) p = 0.003†††	−0.27 (−0.41 to −0.13††) p < 0.001†††	−0.14 (−0.29 to 0.02††) 0.098†††
Number needed to treat ††††	4 (2 to 12)	3 (2 to 8)	NA

*Positive outcome assigned to children missed at follow-up in the MD arm and negative outcome assigned to those missed at follow-up in the CT arm.
**Negative outcome assigned to children missed at follow-up in the MD arm and positive outcome assigned to those missed at follow-up in the CT arm.
†Exact (Clopper-Pearson) 95% CI
††95% CI calculated using binomial regression
†††p-value obtained from Pearson's Chi-square as per study design

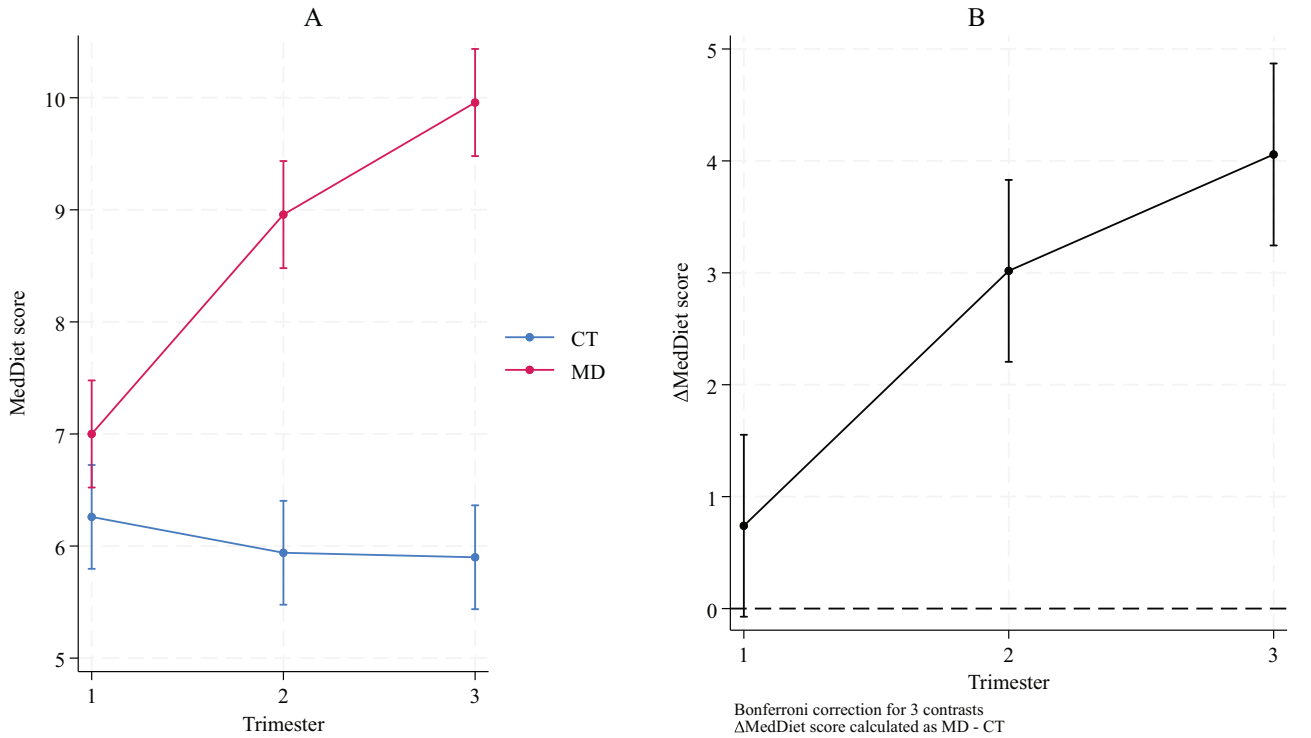

Fig. 2 Changes of the MedDiet score at the 1st, 2nd and 3rd trimester of pregnancy. A plots the changes of the MedDiet score in the MD and CT arms at the 1st, 2nd, and 3rd trimester of pregnancy (PPA). **B** plots three Bonferroni-corrected between-group (MD vs. CT) weight change within-time (1st, 2nd and 3rd trimester) contrasts. Values are means and 95% CI obtained by random effects linear regression.

weight. The same conclusions were drawn using baseline BMI as covariate instead of baseline weight (RELR estimates not shown).

Genome-wide DNA methylation analyses

To assess whether specific DNA methylation signatures are associated with MD during pregnancy, we analyzed the genome-wide methylome using the Infinium EPIC array, which evaluates the methylation status of 850000 probes. To this aim, we randomly selected 11 women from the MD arm and 11 from the CT arm. First, we evaluated whether MD women differed from CT women at the epigenome-wide level. To this aim, we performed PCA by clustering samples according to the rate of methylation at single CpG sites, genes, and promoters' levels (Fig. 3A–C, respectively). In all analyzed regions and CpG sites, the analysis of whole methylome profiles gave comparable results in the MD and CT women. This may be partially explained by the high inter-individual variability of women belonging to each group and by the small number of women ($n = 22$). We then performed hierarchical clustering based on the methylation levels at sites or regions with the highest variance across all samples. By this way, we selected CpGs, promoters and genes that may be more discriminant between the MD and CT groups. Also in this case, we did not observe differences between the two groups (Fig. 3D–F).

Since the leptin gene is the principal gene associated with obesity, we analyzed DNA methylation at the promoter region of the leptin gene in MD and CT women with a high-resolution approach through amplicon-bisulphite sequencing. We analyzed a region of 317 base pairs comprising 28 CpG sites. The mean (SD) methylation rate of the leptin gene in cord blood was 30.4 (1.02) % and 16.9 (2.99) % in the MD and CT mothers, respectively ($p < 0.0001$) (Fig. 4A). Furthermore, we observed in all the analyzed CpG sites of the MD arm higher methylation levels compared to CT arm (Fig. 4B).

DISCUSSION

The current epidemic of pediatric obesity requires effective preventive measures [25]. Nutrition is a major modifiable factor that can influence the development of disease throughout the lifespan [26]. Nevertheless, the effects of nutritional exposures during the prenatal period are likely to be the deepest, resulting in long-term phenotypic changes in the offspring [7, 27]. Therefore, focusing on the maternal diet during pregnancy may be a viable approach for preventing overweight and obesity later in life. The MD is considered a healthy dietary pattern and has been associated with a lower risk of non-communicable diseases, including obesity [28]. In particular, MD during pregnancy has been proposed as a potential dietary strategy to prevent overweight or obesity in children [29].

Previous prospective and retrospective observational studies have yielded conflicting results about the effects of MD in pregnancy on the prevention of overweight or obesity in the offspring. A study of 1827 mother-child pairs from the Spanish "Infancia y Medio Ambiente" cohort reported no association between MD during pregnancy and childhood overweight and obesity. However, there was an inverse association between adherence to MD and waist circumference, a surrogate measure of abdominal obesity [30]. On the contrary, a study analyzing 997 mother-child pairs from "Project Viva" in Massachusetts (USA) and one of 569 pairs from the "Rhea study" in Crete (Greece) showed that maternal MD in pregnancy was associated with lower BMI standard deviation scores in children aged 4 to 10 years [9]. The "NEST" cohort study, including 929 mother-child pairs, reported that higher adherence to MD during pregnancy was associated with lower body size at birth and that such effect was maintained to ages 3 to 5 and 6 to 8 years in the offspring [31]. Lastly, in a cross-sectional study that looked at how dietary counseling helps pregnant women with obesity, it was found that adherence to MD was associated with reduced

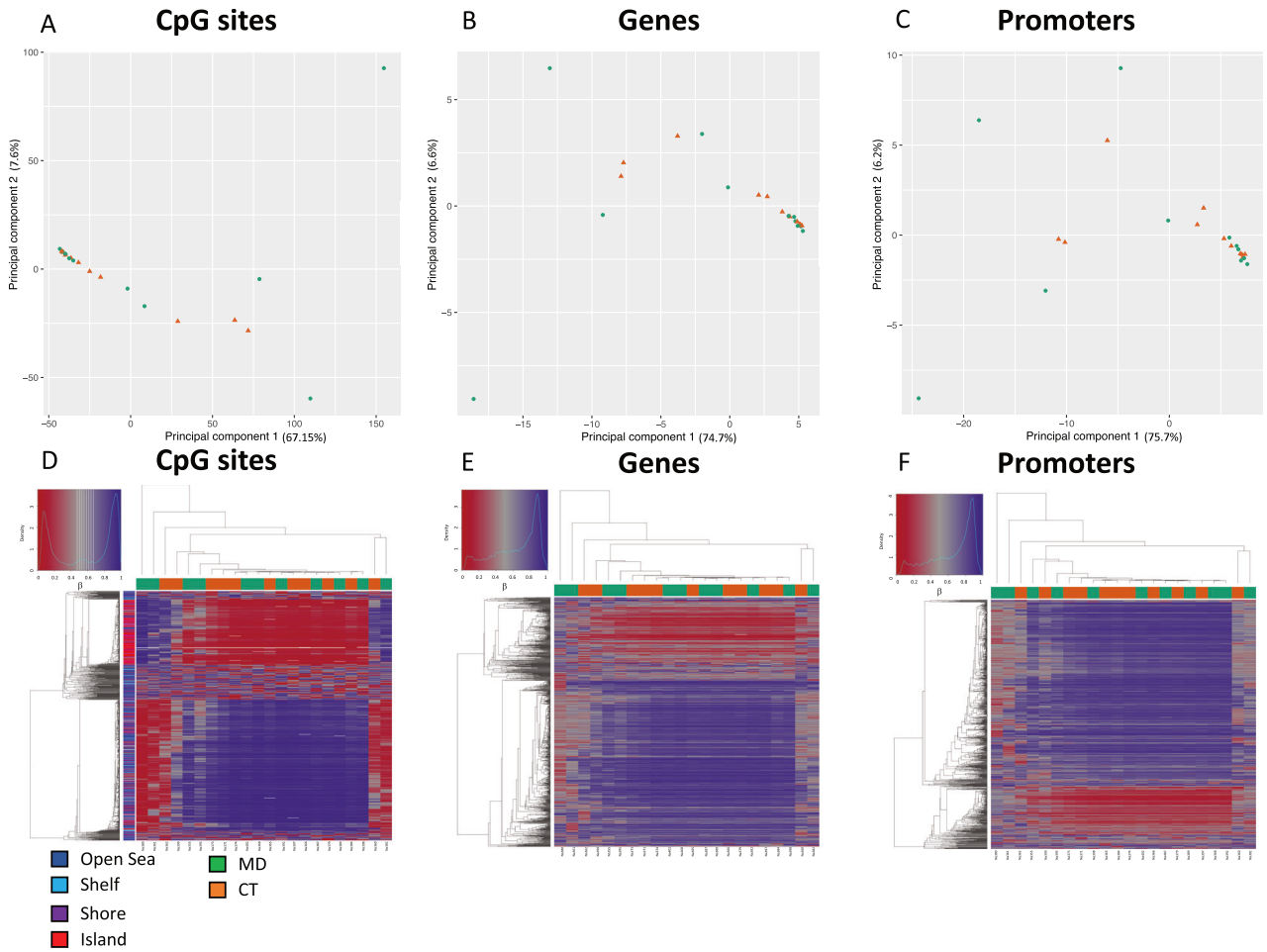

Fig. 3 Epigenome-wide analysis between MD and CT arms. A–C show the principal component analysis for MD ($n = 11$) and CT ($n = 11$) arms. The plots are shown considering the DNA methylation levels at CpG sites (**A**), genes (**B**) and promoters (**C**). **D–F** represent the hierarchical cluster for MD ($n = 11$) and CT arms ($n = 11$). Heatmaps show the methylation profiles at selected sites/regions with the highest variance across all samples.

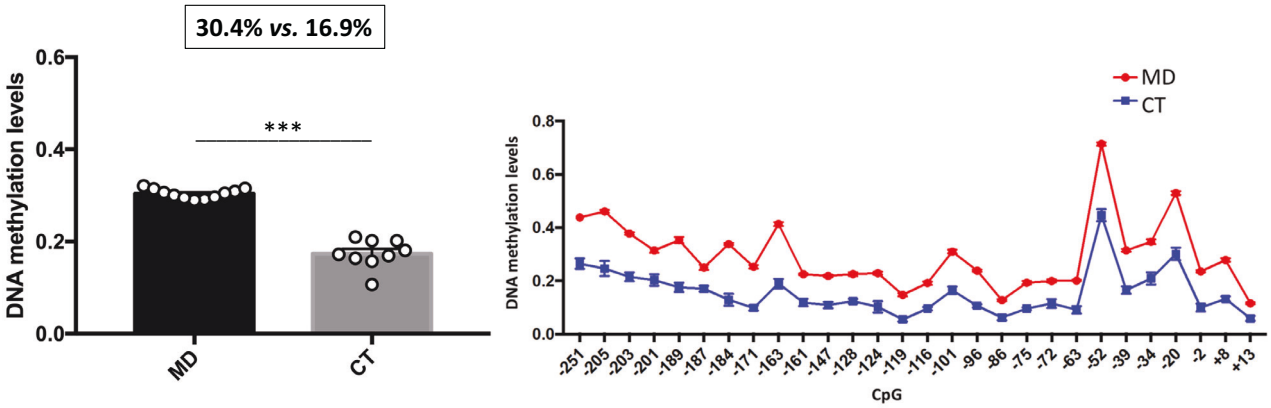

Fig. 4 Average methylation and DNA methylation at each analyzed CpG site at Leptin gene promoter. The numbers of CpG sites are referred to the transcriptional start site (TSS). Values are individual values, means and standard errors. Between-group comparisons were performed using the Student's t test. *** $= p < 0.0001$.

gestational weight gain, newborn birth weight, fat mass, and cord leptin level [10].

The lack of RCT aimed at evaluating the effects of MD nutritional counseling during pregnancy on overweight or obesity in the offspring is a major limitation for understanding the role of MD in overweigh/obesity prevention. The purpose of the PREMEDI trial was to evaluate the effects of MD nutritional counseling during pregnancy on the occurrence of overweight or obesity in the offspring. We found that adherence to MD at baseline was low in all women. Our findings agree with previous studies, showing that

MD adherence during pregnancy is generally poor [32–34]. However, our findings suggest that personalized nutritional counseling could be effective in improving MD adherence during pregnancy, as reported in studies on adults highlighting how dietary guidance is crucial for enhancing MD adherence [35].

Among the mechanisms by which MD in pregnancy may affect fetal programming and exert long-term protective effects on the offspring are epigenetic mechanisms [6, 36]. Maternal diet leads to specific epigenetic signatures that may predispose to the development of late-life obesity [37]. Adherence to MD has been associated with increased maternal gut microbial diversity, promoting the abundance of beneficial metabolites and bacteria able to modulate epigenetic mechanisms [38]. The high intake of plant foods, such as whole grains, legumes, vegetables, and fruits, is the key component driving this association [39]. The production of these microbial compounds, such as butyrate, folate, and biotin, may epigenetically regulate the energy balance and the metabolism of substrates, potentially resulting in an anti-obesity effect [40, 41].

In the present RCT, the risk of overweight or obesity was much lower in the children of mothers enrolled in the MD arm than in the CT arm. This effect was associated with a higher DNA methylation rate of the leptin gene in cord blood mononuclear cells, suggesting a reduction in gene expression. Leptin is a growth hormone that regulates appetite, metabolism, and body fat distribution. Its synthesis occurs in the placenta, and its cord blood levels are associated with newborn anthropometry and body fat [42, 43]. The observed increase in methylation at the leptin gene promoter region suggests that the expression level of this obesity-related gene may be lower in women following MD. Our results are in line with previous observation reporting low levels of leptin in the cord blood of women following MD during pregnancy [10].

Even if PREMEDI was the first RCT to evaluate the effects of MD nutritional counseling during pregnancy on the incidence of overweight or obesity in the offspring, the study presents limitations. Given the nature of the treatments, it was not possible to blind the participants. In addition, the relatively small sample size, the lack of evaluation of other biomarkers of MD adherence, and the limited number of samples evaluated for the genome-wide DNA methylation analysis could limit the generalizability of the study results.

CONCLUSION

In summary, nutritional counseling aimed at promoting MD adherence during pregnancy may protect the offspring against overweight or obesity at the age of 24 months. This effect may be partly mediated by an epigenetic modulation of leptin expression. Our findings support the role of MD during pregnancy as a safe, effective, and potentially cost-saving strategy against the pediatric obesity pandemic.

DATA AVAILABILITY

The data underlying this article will be shared on reasonable request.

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AUTHOR CONTRIBUTIONS

SC, LP, RN, and RBC: study design; SC, LC, AA, MN, FM, AP, and RBC: data acquisition; GB: statistical analysis of clinical outcomes; LP, DC, MC, and LC: laboratory analysis and statistical analysis of laboratory outcomes; SC, LP, GB, MC, and RBC: drafting of manuscript; all authors critically reviewed and approved the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The trial was approved by the Ethics Committee of the University of Naples Federico II and was conducted in accordance with the Helsinki Declaration and relevant European and Italian privacy regulations. Written informed consent to participate in the study was obtained from the women and no financial compensation was provided. The trial was registered on ClinicalTrials.gov (Identifier: NCT03337802). The study took place at the Villa Betania Evangelical Hospital in Naples, Italy, between November 30, 2017 and January 31, 2021. The study is reported following the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

ADDITIONAL INFORMATION

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