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Relationships between the maternal prenatal diet and epigenetic state in infants: a systematic review of human studies

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Review

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Abstract

Most human studies investigating the relationship between maternal diet in pregnancy and infant epigenetic state have focused on macro- and micro-nutrient intake, rather than the whole diet. This makes it difficult to translate the evidence into practical prenatal dietary recommendations.

To review the evidence on how the prenatal diet relates to the epigenetic state of infants measured in the first year of life via candidate gene or genome-wide approaches.

Following the PRISMA guidelines, this systematic literature search was completed in August 2020, and updated in August 2021 and April 2022. Studies investigating dietary supplementation were excluded. Risk of bias was assessed, and the certainty of results was analysed with consideration of study quality and validity.

Seven studies were included, encompassing 6852 mother-infant dyads. One study was a randomised controlled trial and the remaining six were observational studies. There was heterogeneity in dietary exposure measures. Three studies used an epigenome-wide association study (EWAS) design and four focused on candidate genes from cord blood samples. All studies showed inconsistent associations between maternal dietary measures and DNA methylation in infants. Effect sizes of maternal diet on DNA methylation ranged from very low (< 1%) to high (> 10%). All studies had limitations and were assessed as having moderate to high risk of bias.

The evidence presented here provides very low certainty that dietary patterns in pregnancy relate to epigenetic state in infants. We recommend that future studies maximise sample sizes and optimise and harmonise methods of dietary measurement and pipelines of epigenetic analysis.

Introduction

The Developmental Origins of Health and Disease (DOHaD) hypothesis contends that environmental factors, such as maternal nutrition, stress and infection, during fetal development can influence the short and long-term health and non-communicable disease (NCD) status in offspring.[1,2]

Experimental animal studies support the DOHaD hypothesis[3] and the role of maternal nutrition.[4–7] Pre-clinical studies have shown that components of maternal nutrition such as fat intake (e.g., fish oil and omega-3 polyunsaturated fatty acids (omega-3 PUFA)) can affect gene expression, body weight, glucose tolerance and insulin sensitivity in offspring.[4–7] However, evidence in humans is limited and mainly in the form of observational studies.[8]

One of the proposed DOHaD mechanisms is via alteration in gene expression through epigenetic modification.[2] Epigenetics describes the molecular mechanisms that control gene activity without changing the DNA sequence.[9] During early development, differentiation is accompanied by changes in epigenetic state, which are inherited when cells divide, thus acting as cellular memory. Epigenetic state is controlled by a combination of stochastic, genetic and environmental factors, the latter including internal, such as hormone signalling, and external, such as nutrition. Epigenetic modifications include DNA methylation, histone modification and non-coding RNA, which together, regulate gene activity. The most widely studied form of epigenetic modification is DNA methylation, specifically at the cytosine of a cytosine-guanine (CpG) dinucleotide. Microarray and sequence-based DNA methylation profiling are two technologies that are broadly used to study methylation status. The way in which methylation status is differentiated can be via restriction enzyme digestion, methyl-binding antibodies or proteins or through bisulphite conversion of genomic DNA.[10]

Diet can influence epigenetics via the one-carbon metabolism pathway which is one of the main metabolic networks allowing nutrients to modulate DNA methylation.[11] On this basis,

many studies have investigated the relationships between folate supplementation and DNA methylation (reviewed by).[12] However, more broadly, dietary intake of folate, choline, betaine, methionine and B group vitamins have been implicated in global methylation and methylation in promoters of disease-specific genes in both animal and human studies.[12]

A small number of animal studies have examined the relationship between macronutrients in maternal diet during pregnancy and epigenetic state in offspring.[13,14] Rodent studies have focused on the effects of supplementation with vitamins such as folic acid, protein and fat on DNA methylation levels in fetal to pre-weaning stages. Evidence from rat models indicate that protein restriction throughout pregnancy results in either global or locus-specific changes in DNA methylation.[15] Furthermore, a study of methyl-deficient rats in prenatal, postnatal and dietary transition periods suggested that responsiveness of the nutritional change is tissue specific.[16]

Two systematic reviews have investigated the relationship in humans between prenatal maternal dietary supplements and DNA methylation in newborns.[17,18] The first review included eight human studies, three intervention studies that trialled folic acid supplementation and five which were observational.[19] Studies were heterogeneous in design and included candidate and genome-wide approaches. Some studies identified folic acid supplements associated with differential methylation,[20–22] while others did not.[23] An important finding was that in a folate-replete population, excess intake of folate is likely to impact global (genome average) DNA methylation in infants.[17] The second systematic review identified that nutritional supplementation with vitamins and micronutrients during pregnancy had little effect on offspring DNA methylation unless the results were stratified by sex, BMI and prenatal smoking.[18] However, experimental approaches primarily based on candidate genes and global levels of DNA methylation, were heterogeneous in design and no consistent effects have been found.[24] The authors called for further studies investigating single versus multiple micronutrient supplementation.

In addition to dietary supplements, we propose that *full dietary intake* should be considered in studies investigating the effects of maternal nutrition on epigenetic state of offspring in humans. The disadvantages of investigating DNA methylation in relation to prenatal micro- and macro-nutrient intake are that this may not capture important interactions between foods, and it becomes difficult to translate the evidence to practical dietary recommendations for women to follow. Only by investigating dietary intake, or proxies for dietary intake, can the dietary consequences on the infant epigenome be assessed. Ultimately, such data could be linked with offspring health, and used to strengthen evidence for prenatal dietary recommendations. Equitable access to dietary supplements is not guaranteed in any country. Therefore, as a complement to the current evidence, an understanding of how diet influences DNA methylation in humans could help establish healthy dietary patterns that are easy to follow. This is particularly important given the present nutritional landscape, where more support is needed to help women meet the dietary recommendations.[25]

Therefore, the aim of this systematic review is to synthesise current knowledge on how the maternal prenatal diet relates to the epigenetic state of infants. We aim to review the associations between specific dietary patterns in pregnancy and epigenetic state in infants, measured via candidate gene or genome-wide approaches. The results of this review will help to identify whether aspects of the prenatal diet or full dietary intake are associated with epigenetic state in offspring.

Methods

Search strategy

This systematic review was conducted according to PRISMA guidelines.[26] We performed a systematic literature search between July 2020 and August 2020 and performed a search update in August 2021, a final hand search was conducted in April 2022. The PubMed, CINAHL, Ovid MEDLINE and Embase databases were searched using a predefined, structured search strategy aiming to retrieve human studies investigating the maternal prenatal diet and maternal and offspring epigenetic regulation. The following five search groups were combined using an 'and' operator, (1) for diet: 'food OR nutrition OR diet OR macronutrient OR nutrient OR Mediterranean diet OR vegetarian OR vegan OR omnivore OR restriction diet OR dietary pattern'; (2) for epigenetics: 'epigenetic OR epigenome OR methylation OR histone OR noncoding RNA OR acyltransferase'; (3) for mothers: 'mother OR maternal OR pregnant OR pregnancy OR motherhood OR perinatal'; (4) for infants: 'progeny OR prenatal OR baby OR infant OR neonate OR neonatal OR offspring'; (5) for human studies: 'randomized controlled trial OR RCT OR rct OR observational study OR cohort study OR case-control study'.

Study selection, inclusion and exclusion criteria

In this review, the chosen population was pregnant women where diet was measured as an exposure and epigenetic outcomes were measured in infants. Experimental studies comprising double-blind, randomised, placebo-controlled clinical trials and observational studies comprising cohorts and longitudinal studies were included. These studies involved measurement of food intake through diet, dietary components or proxies for dietary components. The search was limited to human studies written in English. Studies were excluded if there was: use of nutritional supplementation interventions without a measurement of dietary intake; a lack of reporting of epigenetic outcomes for mother or infant; a lack of measurement or report of aspects of maternal dietary intake; or incorrect timeframe i.e. maternal diet prior to conception or epigenetic outcomes measured during toddler stages (from around one year of age) or later. Studies were screened for inclusion and exclusion by one author (KF), study eligibility was reviewed by all authors using Rayyan,[27] and disagreements were resolved through discussion.

Data extraction

Two reviewers independently collected data from each study (KF & JC), these data were checked by KF, and any disagreements were resolved through discussion with all authors. No automation tools were used to collect or translate data. The following data were extracted: study design, study aim, setting, year, participant characteristics, the exposure conditions and duration, study primary and secondary outcomes, epigenetic outcome measure, analysis methods and technology, study results and model

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adjustments. We inferred the 'primary' and 'secondary' outcomes based on the study's aim and design and when these were not explicitly stated in a study.

Critical appraisal assessment

We devised an assessment rubric to appraise the methodological quality and validity of the epigenetic data analysis methods used. This rubric evaluated design aspects such as the sample size, scale of epigenetic analysis, whether best-practice methods and results were reported, and whether the results were described fair and accurately in the abstract. This appraisal informed the evidence certainty assessment (GRADE)[28] for study limitations, imprecision and inconsistency.

Assessment of risk of bias/study quality

The risk of bias was assessed using the Jadad scale[29] for randomised controlled trials and the quality of non-randomised trials was assessed using the Newcastle-Ottawa scale.[30] The risk of bias or quality (as appropriate) in each study was classified as low, medium or high. Two authors (KF and SD) assessed the risk of bias independently and disagreements were resolved through discussion. Robvis was used to generate risk of bias plots.[31] Reporting bias was assessed with consideration of the results presented and magnitudes of effect.

Certainty assessment

GRADE[28] was used to assess the certainty of results for the association between specific dietary patterns in pregnancy and epigenetic state in infants (measured via candidate gene or genome-wide approaches) with consideration of the study quality and validity i.e. study limitations, imprecision, indirectness, consistency of effect and risk of bias.

Results

Study selection and study characteristics

Our search identified 771 records (Fig. 1); from these, 251 were excluded as duplicates and 520 were subjected to screening for eligibility. A total of 479 records were excluded as the titles were not relevant to either diet or epigenetics, focused on micronutrients and/or supplementation or were review articles. Abstracts of the remaining 41 articles were assessed for eligibility and 35 were excluded, leaving five eligible studies.[32–36] An updated search conducted 12 months after the initial search yielded no new studies. In 2022 April, two further articles were identified through hand-searching selected references, yielding a total of seven eligible studies for the systematic review[32–38] (Table 1). Only one study was a randomised controlled trial[32]; the remaining six were observational studies,[33–38] one of which pooled DNA methylation data across five large birth cohorts.[38]

Exposure measures

There was considerable heterogeneity in dietary exposure measures. Three out of the seven studies used measures of fat intake; specifically, dietary omega-3 PUFA,[35] oily fish,[36] and fats [37] (Table 1). Three studies measured specific dietary patterns, a low glycaemic index[32] and the Mediterranean diet.[34,38] In another study, the exposure was maternal calorie intake.[33] Dietary intake measurement differed between studies and included food diaries,[32] food frequency questionnaires,[33,34,36–38] 24-hour recall,[38] and biomarkers of omega-3 PUFA from maternal blood 12-24 hours prior to birth.[35] The exposure duration varied, ranging from the time of study enrolment during gestation until completion of the second,[37] or third trimester.[32–34,36,39] In the large PACE consortium study, the exposure duration varied across the five cohorts.[38]

Outcome measures

None of the included studies reported methylation outcomes for mothers. Six of the seven studies reported infant DNA methylation as the primary (main) study outcome.[32–38] One study reported DNA methylation as a secondary outcome to child behavioural outcomes[34] (Table 1). The scale of DNA methylation analysis differed between studies; three used an epigenome-wide association study (EWAS) design conducted on 485,000[35,38] and 850,000[32] CpGs, while four studies focused on a small number (1-8) of candidate genes and their differentially-methylated CpGs (DMCpGs) or regions (DMRs).[33,34,36,37]

Secondary analyses were performed within some studies including replication analysis of top candidate genes derived from the primary EWAS; association of DNA methylation with gene expression and genetic polymorphisms[36]; and association between fat intake and DNA methylation at the insulin-like growth factor 2 (*IGF2*) differentially methylated region (DMR) and the *H19*-DMR, birth weight for gestational age and cord blood levels of IGF2 protein.[37]

Six studies used umbilical cord blood white blood cells (WBC) for the infant tissue sampled for epigenetic analysis,[33,34,36–38] and one used cell-free DNA extracted from cord blood serum.[32] DNA methylation was measured using either Illumina methylation arrays,[32,35,36,38] bisulphite pyrosequencing[34,37] or high-resolution melt analysis.[33]

Study results

All seven studies included in this review showed inconsistent associations between maternal dietary measures and DNA methylation in infants (Table 2). The effect sizes ranged from very low (<1%),[32,34,36,38] low (1%–5%), moderate (5%–10%),[37] to high (>10%)[33] and were not reported in one study.[35]

Gene-specific studies

The C-MACH study reported lower DNA methylation in offspring of large effect sizes (Table 2) in the H19-DMR in offspring of mothers with low-calorie intake compared to those with medium and high-calorie intake, respectively.[33] The NEST study reported sex-dependent variations in methylation across six imprinted genes in offspring, varying as maternal adherence to a Mediterranean dietary pattern increased, albeit with very low effect sizes. The Project Viva study reported higher and lower methylation of moderate effect size within the *IGF2* and *H19* genes in offspring of mothers with exposures related to different types of fat. Only one of the four gene-specific studies adjusted for multiple testing. Three of the studies adjusted models for relevant covariates, e.g. infant sex or gestational age at delivery.[33,34,36,37] In the Isle of Wight 3rd Generation Cohort, *FADS1/2* and *ELOVL5* were selected from the methylation array data as candidate genes. It also reported higher and lower methylation of low/very low effect size in the *FADS1/2* and *ELOVL5* genes (respectively) in offspring of mothers with higher intake of oily fish.

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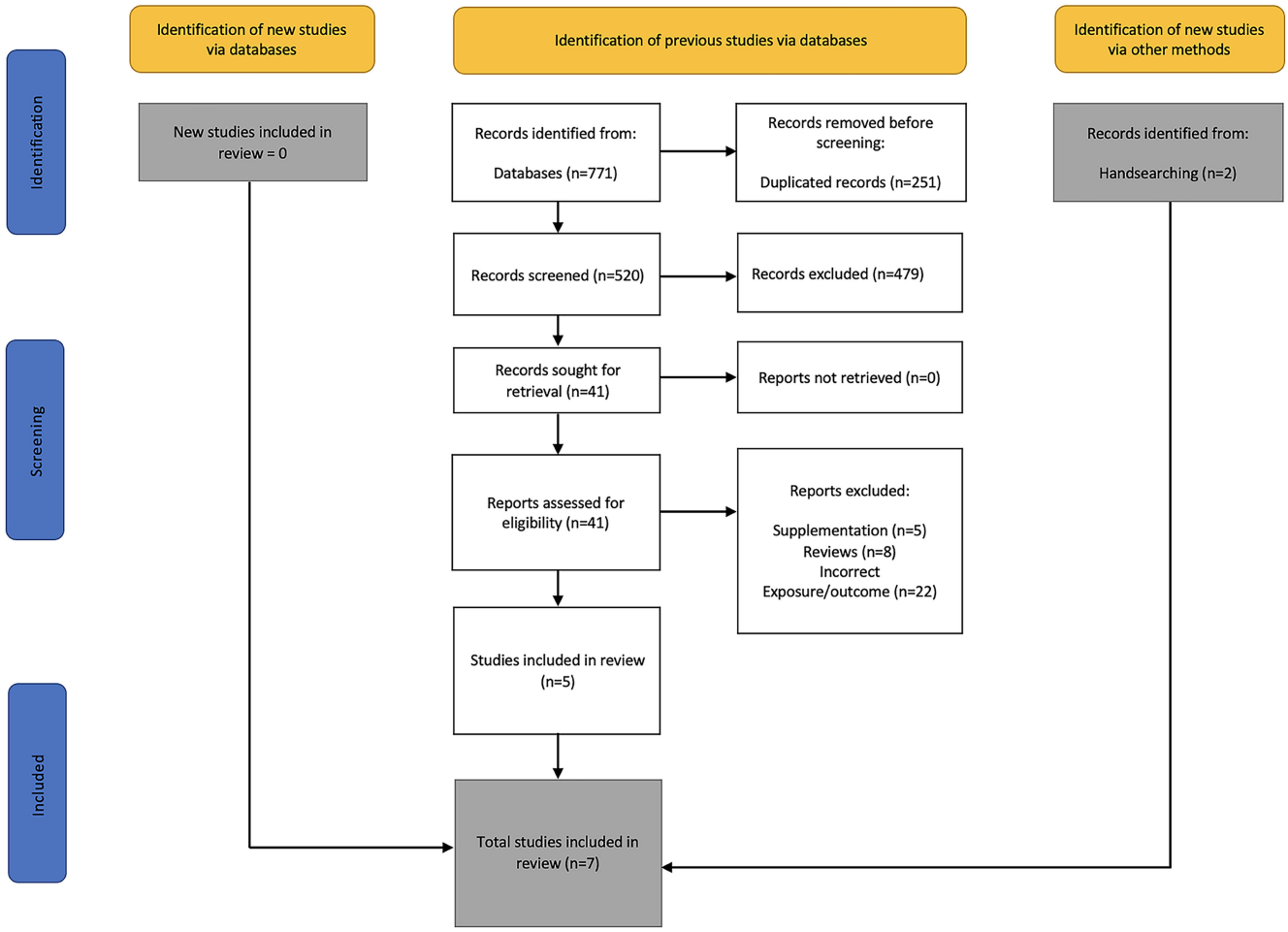

Figure 1. PRISMA flow diagram showing selection of included studies for this review.

Genome-wide studies

The ROLO RCT study reported higher methylation with a very low average effect size (Table 3) across all probes in offspring of mothers who consumed a low glycaemic index diet in comparison to the controls, and higher methylation with a low effect size for the same comparison in the 1000 probes ranked by *p*-value. The 'Feeding fetus' study did not provide the direction or effect size in DNA methylation in a region between the *COX19* and *ADAP1* genes in offspring of mothers with the highest tertile of intake of omega-3 PUFA compared to the lowest tertile. In the PACE consortium, one *WNTB5B* CpG had a significantly higher level of DNA methylation associated with maternal adherence to the Mediterranean diet. Of the four genome-wide studies, two adjusted for multiple testing and adjusted for relevant covariates,[38] while another used a 'combined' unadjusted method,[35] and another performed no model adjustment.[36]

Critical appraisal assessment

The methodological quality and validity of the epigenetic data analysis varied across studies (Table 3). The sample sizes were generally small (<100 per group) or moderate (100–1,000 per group). There was consistency in the sample tissue used, with all but one study[32] using DNA from cord blood WBC samples. However, these authors used cell-free DNA from cord blood, which is likely to have also originated from WBCs. As DNA methylation levels differ in part between tissues, this strengthens the quality of evidence in this systematic review. There was heterogeneity in the approach used for epigenetic analysis, which may explain the inconsistencies. For example, three studies used EWAS,[32,35,36,38] three studies used candidate genes and CpGs[33,34,36,37] and another study used both approaches.[40]

Data quality control steps, such as removal of poor-performing samples, removal of probes hybridising to multiple genomic locations[41,42] and normalisation across probes, were described in three out of six studies, leading to imprecision in the results (Table 3). Only the 'Feeding fetus' study did not provide the number of CpGs used following initial QC in EWAS analysis whereas it was provided consistently in the rest of the studies. Some studies did not adjust for potential confounders such as cellular heterogeneity, sex and age at delivery. Only the Isle of Wight 3rd Generation cohort study[40] investigated the influence of genetic influence on epigenetic state, which can also confound studies if genotypes are unevenly distributed between groups.[43] This added to the inconsistency and imprecision. There was also inconsistent use of the methods used to adjust for multiple testing, which weakens interpretation due to the higher likelihood of type 1 errors.

Both EWAS studies did not list the highest-ranking DMCpGs/DMRs along with their effect sizes and p-values. This information is essential to judge the strength of association and omission

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Table 1. Study characteristics

Study design	Randomised controlled trial	Cohort studies					
Study	ROLO study Geraghty et al. (2018)	C-MACH Miyaso et al. (2017)	NEST House et al. (2018)	'Feeding fetus' low-grade inflammation and insulin resistance study Bianchi et al. (2019)	Isle of Wight 3rd Generation Cohort Losol et al. (2019)	Project Viva Chiu *et al.* (2021)	PACE consortium Küpers et al, 2022
Cohort	The Randomised control trial of low glycaemic index diet to prevent macrosomia (ROLO) study[76]	The Chiba study of Mother and Children's Health (C-MACH) study was designed to investigate effects of genetics and environmental factors on children's health[77]	The Newborn Epigenetics Study (NEST) Study was designed to identify early exposures associated with epigenetic alteration[21]	The Feeding Fetus study was designed to investigate the relationship between maternal lipid intake with offspring inflammation and insulin resistance at birth[78]	The Isle of Wight 3rd Generation Cohort study was designed to identify effects of epigenetic processes in transgenerational inheritance[79]	Project Viva was designed to identify associations between prenatal intake of different types of fat in trimesters 1 and 2 pregnancy on DNA methylation at birth[37]	PACE is consortium of five birth cohorts designed to study the relationship between maternal environment and epigenetic state in children (Küpers et al, 2022)
Study setting and years	Dublin, Ireland 2007–2011	Chiba, Japan 2014–2019	Durham, North Carolina 2009–2011	Rome, Italy 2013–2015	Isle of Wight, UK 2010–2018	Eastern Massachusetts, USA 1999–2002	UK, The Netherlands, Spain & USA NS
Number of mother-infant pairs in the study (number in the DNA methylation analysis dataset)	60 (60)	101 (91)	325 (142)	1000 (118)	436 (114)	2128 (96)	(2802)
Participant characteristics	Women in their second pregnancy who had previously given birth to a macrosomic infant and over 18 years	Pregnant women who did not smoke, were not treated for infertility, and did not experience preterm or post-term delivery	Pregnant mothers 18 years or older and excluded those with HIV	Women between 18–39, having a healthy a planned singleton pregnancy, with folic acid supplementation	NS	Pregnant women without a history of gestational diabetes, pre-eclampsia, unplanned pregnancy, who delivered between gestation week 40-42	Mother and child pairs with complete data for maternal Mediterranean diet score, DNA methylation and covariates
Dietary exposure conditions and/or measures	Intervention condition starting before 18th week of gestation to end of pregnancy included dietary advice relating to a low glycaemic index diet during pregnancy ($n = 30$). Control condition was no specific dietary advice during pregnancy ($n = 30$)	Maternal daily caloric intake was divided into 3 groups: low calorie < 1000kcal/day (n = NS), Middle calorie, 1000–1999 kcal/day (n = NS), and high calorie, >2000 kcal/day (n = NS)	Maternal Mediterranean diet adherence-MDA (continuous measure) with scores ranging from 0 to 9, where 9 was most adherent	omega-3 PUFA intake tertiles: Low ($n = 39$), Medium ($n = 40$), High ($n = 39$)	Intake frequency of white fish, shellfish, and oily fish consumption was dichotomised into low consumption, uncertain, never, or 1–2 or less per month ($n = 103$), or high, 1 or more per week ($n = 11$)	Fatty acid intake as a continuous variable using the multivariable nutrient density model including total energy, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, omega-3, omega-6 and trans-fat intake	Maternal adherence to the Mediterranean diet during pregnancy using the relative Mediterranean diet (rMED) score, tertiled
Dietary exposure	Intervention condition starting before 18th week of	Maternal daily caloric intake was	Maternal Mediterranean	omega-3 PUFA intake tertiles: Low ($n = 39$),	Intake frequency of white fish, shellfish, and oily	Fatty acid intake as a continuous variable using	Maternal adherence to the Mediterranean diet

Table 1. (*Continued*)

conditions and/or measures	gestation to end of pregnancy included dietary advice relating to a low glycaemic index diet during pregnancy ($n = 30$). Control condition was no specific dietary advice during pregnancy ($n = 30$)	divided into 3 groups: low calorie < 1000kcal/day ($n =$ NS), Middle calorie, 1000–1999 kcal/day ($n =$ NS), and high calorie, >2000 kcal/day ($n =$ NS)	diet adherence-MDA (continuous measure) with scores ranging from 0–9, where 9 was most adherent	Medium ($n = 40$), High ($n = 39$)	fish consumption was dichotomised into low consumption, uncertain, never, or 1–2 or less per month ($n = 103$), or high, 1 or more per week ($n = 11$)	the multivariable nutrient density model including total energy, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, omega-3, omega-6 and trans-fat intake	during pregnancy using the relative Mediterranean diet (rMED) score, tertiled
Dietary measurement tool, collection timepoint	Three-day food diaries collected in each trimester of pregnancy. Diaries were completed on 3 consecutive days, 2 weekdays and a weekend day	Brief-type self-administered diet history questionnaire (BDHQ) collected during gestation week 12	Food frequency questionnaire collected after conception or around the time the last menstrual period	Biomarker of omega-3 PUFA intake using erythrocyte omega-3 PUFAs expressed as percent weight fraction of the total amount of fatty acids quantified on erythrocyte membranes measured by gas chromatography, collected 12–24 hours prior to birth	Food frequency questionnaire[80] during 24th and 28th week of gestation	Food frequency questionnaires collected during the first trimester and at 26-28 weeks of gestation	Varied across cohorts: Food frequency questionnaires, or 2–3 24-hour recalls
Study outcome measures and sample tissue	Primary: association of exposure with DNA methylation in an EWAS of 771,484 CpGs in cell-free DNA from cord blood serum Secondary: replication analysis of top candidate genes from EWAS – *IL17D*, *NFIC* & *TBCD*. Exploratory: differences in cell composition between intervention and control groups and association of cell types with DNA methylation	Primary: association of exposure with average cord blood WBC DNA methylation within the imprinted H19 gene differentially methylated region (DMR)	Primary: child behaviour Secondary: association of exposure with cord blood WBC DNA methylation at 48 CpGs across of 8 imprinting control regions (ICRs)	Primary: association of exposure with DNA methylation in an EWAS of 450,000 CpGs in cord blood WBC DNA	Primary: association of exposure with DNA methylation at 39 CpGs within the *FADS1/2* genes and 27 CpGs within the *ELOVL5* gene in cord blood WBC DNA. Secondary: association of DNA methylation with gene expression and genetic polymorphisms	Primary: association of exposure with DNA methylation at *IGF2* (2 CpGs & average) and *H19* (6 CpGs & average) DMRs in cord blood WBC DNA Secondary: association between fat intake and DNA methylation at the *IGF2*-DMR and *H19*-DMR and (1) birth weight for gestational age and (2) cord blood levels of IGF2 protein	Primary: association of exposure with DNA methylation in an EWAS of 429,701 CpGs in cell-free DNA from cord blood WBC DNA
Biosample used	Cell-free DNA from cord blood serum	Umbilical cord blood WBCs	Umbilical cord blood WBCs	Umbilical cord blood WBCs	Umbilical cord blood WBCs	Umbilical cord blood WBCs	Umbilical cord blood WBCs
Method of measuring DNA methylation	Illumina Infinium MethylationEPIC BeadChip Arrays; Sequenom MassArray EpiTyper	Methylation-sensitive high resolution melt analysis	Bisulfite pyrosequencing	Infinium HumanMethylation450 BeadChip arrays	Illumina Infinium HumanMethylation450 and MethylationEPIC BeadChip arrays	Bisulfite pyrosequencing	Infinium HumanMethylation450 BeadChip arrays

(*Continued*)

Table 1. (*Continued*)

Study design	Randomised controlled trial	Cohort studies					
Study	ROLO study Geraghty et al. (2018)	C-MACH Miyaso et al. (2017)	NEST House et al. (2018)	'Feeding fetus' low-grade inflammation and insulin resistance study Bianchi et al. (2019)	Isle of Wight 3rd Generation Cohort Losol et al. (2019)	Project Viva Chiu *et al.* (2021)	PACE consortium Küpers et al, 2022
What CpGs or regions were measured	771,484 CpGs	Average CpG methylation across the H19 DMR	Average CpG methylation at 48 CpGs across of 8 imprinting control DMRs	485,000 CpGs analysed as CpGs and DMR	39 CpGs in the *FADS1/2* genes, 27 in *ELOVL5*	Average and individual CpG methylation (2 CpGs in *IGF2*-DMR, 6 in *H19*-DMR)	~485,000 CpGs analysed as CpGs

NS, Not stated.

increases inconsistency and imprecision. None of the studies calculated the proportion of DMCpGs or DMRs at the stated level of significance as a proportion of all measured, something that would have enabled more accurate comparisons across studies and increased precision. Measuring test statistic inflation (lambda) is one way of monitoring the effects of confounding[44] but was not measured in any of the studies. Validation of top-ranking DMCpGs/DMRs using an independent method of DNA methylation analysis, replication in an independent cohort and meta-analysis are methods to reduce type 1 errors and to maximise the likelihood of agreement across studies. However, the ROLO study[32] did attempt replication, although the original findings were not replicated.

Association of function to top-ranked DMCpGs/DMRs using enrichment analysis or analysis of protein and/or RNA levels can provide information about changes to gene function associated with change in DNA methylation. Two studies attempted this; in one,[36] the effect of fat intake was investigated on the gene expression in *FADS1/2 and ELOVL5* and in Project Viva,[37] the protein levels of IGF2 and H19 were measured.

There was inconsistency and imprecision in the way the results were reported in the title or abstract. Some of the authors reported accurately[33–35,37] and the others over-stated their results.[32,36] In summary, we ranked half the studies as poor quality and half as good quality (Table 3). Low sample size, low effect size and missing details largely contributed to this conclusion.

Assessment of risk of bias

Assessment of risk of bias in randomised controlled trial

The RCT[32] scored two out of a maximum of five points where lower is indicative of greater bias in the Jadad scale[29] (Fig. 2). D1 (study described as randomised) had low risk of bias, although randomisation was used in the main study, this sub-analysis selected 60 sex-matched participants (30 in each group), without providing details about the selection process or accounting for all participants. D3 (blinding mentioned) had high risk of bias as there was no mention of blinding, but this study was likely to be a single-blind RCT as participants would have become aware of group allocation when they attended the intervention education session. Moreover, it is unclear whether the investigators were aware of group allocation during the data analysis phase, which may have biased the results hence D4 (appropriate blinding mentioned) scored high risk of bias. D5 (accounted for all withdrawals and dropouts) was unclear.

Assessment of risk of bias in cohort studies

Representativeness of the selected sample

All cohort studies had a moderate quality scoring between five and seven out of a total of nine (Fig. 3). Scoring for D1 (representativeness of the exposed cohort) varied, Miyaso *et al.*,[33] Chiu *et al.*,[37] and Küpers *et al.*[38] selected a subset of participants from their full cohorts for whom questionnaire data were complete, this impacted on representativeness of participant selection (D1) for[33,37]; however, one study still scored one star due the use of five large population-based cohorts across five countries.[38] In another study,[33] subsetting led to uncertainty around how representative the outcome is of the full cohort as outcome data were not included for 73% of the full study participants (D9-adequacy of follow-up of cohorts) Chiu *et al* also excluded participants due to gestational diabetes, unplanned pregnancy, delivery before 40 weeks or after 42 weeks, although

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Table 2. Epigenetic results

	Randomised controlled trial	Cohort studies					
Study result	ROLO study Geraghty *et al.* (2018)	C-MACH Miyaso *et al.* (2017)	NEST House *et al.* (2018)	'Feeding fetus' low-grade inflammation and insulin resistance study Bianchi *et al.* (2019)	Isle of Wight 3rd Generation Cohort Losol *et al.* (2019)	Project VivaChiu *et* al. (2021)	PACE consortium Küpers *et al.* (2022)
Multiple testing adjustment	FDR < 0.05	Unadjusted $p < 0.05$	Bonferroni	'Combined' unadjusted $p < 0.05$	Unadjusted $p < 0.05$	Unadjusted $p < 0.05$	Bonferroni ($p < 1.16 \times 10–7$)
Statistical model adjustment	Array, chip position, sex, gestational age	Maternal age, height, weight, gestational age calorie intake, carbohydrate intake, serum nutrient concentration and paternal age	Mothers' age at delivery, education level, pre-pregnancy BMI, smoking, gestational diabetes, fibre, folate & energy intake. Models were stratified by sex	Not done	Not done	Pre-pregnancy BMI, total gestational weight gain, infant sex, gestational age at delivery, mode of delivery, mother's age at enrolment and annual household income	Maternal education, age, smoking, pre-pregnancy BNMI, energy intake; infant sex; array batch (in four different combinations)
DNA methylation effect size(s) and genes in offspring for adjusted model	Average effect size in offspring of low maternal glycaemic index mothers was 0.4% higher across all probes and 2.4% higher across the 1,000 top-ranked probes	*H19* DMR was 10.3% lower in offspring of mothers with low calorie intake compared to moderate calorie intake, and 11.18% lower compared to high calorie intake	*SGCD/PEG10* ICR 1.65% higher in offspring per unit increase in maternal adherence to the Mediterranean dietary pattern in females, *IGF2* DMR 0.92% lower; SGCD/*PEG10* ICR 0.56% lower); *PLAGL1* DMR 1.1% lower; *MEG3* DMR 0.97% lower in males	*COX19* & *ADAP1*: effect size not provided	*FADS1/2*, 0.9% lower in cg00614641, 1.0% lower in cg07999042, 0.3% higher in cg12517394; ELOVL5, 1.1% higher in cg10410213, 4.5% higher in cg11748354, 1.3% higher in cg24524396 in offspring of mothers with any fish intake compared to no fish intake	7 exposures, 2 timepoints, 8 individual CpGs, 2 average CpGs within 2 DMRs (IGF2 & H19) = 140 analyses, of which 28 (20%) were significant at $p < 0.05$. Effect sizes -5.1 to 6.9 % per unit change in fatty acids (at expense of carbohydrate) in mothers	In one out of four models, one significant CpG identified at *WNT5B* 0.064% increase in DNA methylation per 1-point increase in the rMED score (range 0–16)

DMR, differentially methylated region; DMCpG, differentially methylated CpG; FDR, false discovery rate; ICR, imprinting control region.

Table 3. Assessment of epigenetic data analysis: methods, quality control

	Randomised controlled trial	Cohort studies						
Assessment criteria	ROLO study Geraghty *et al.* (2018)	C-MACH Miyaso *et al.* (2017)	NEST House *et al.* (2018)	'Feeding fetus' low-grade inflammation and insulin resistance study Bianchi *et al.* (2019)	Isle of Wight 3rd Generation Cohort Losol *et al.* (2019)	Fat intake study Chiu et al. (2021)	PACE consortium Küpers et al, 2022	Grade criteria
Sample size	Small	Moderate	Moderate	Moderate	Small	Small	Large	- Low to moderate sample sizes
Cord blood white blood cell samples	x	✓	✓	✓	✓	✓	✓	+ Consistency across observational studies & RCT likely to be from similar cell types
Scale of epigenetic analysis	EWAS	Candidate genes	Candidate genes	Candidate genes via EWAS	EWAS	Candidate genes	EWAS	- Inconsistency: heterogeneity of approach (candidate vs EWAS) & heterogeneity of methods within each of these
Initial data QC	✓	x	x	✓	✓.	x	✓	- Imprecision: insufficient QC of EWAS
Provided the number of CpGs (probes) used in the analysis following initial QC	✓	✓	✓	x	✓	✓	✓	- Consistency of reporting across most studies
Testing and/or adjustment for potential confounders	✓	✓	✓	x	x	✓	✓	- Imprecision: confounders not measured or adjusted for
Tested for genetic confounding	x	x	x	x	✓	-	✓	- Inconsistency and imprecision

Table 3. (*Continued*)

Adjustment for multiple testing	✓	✓	✓	✓	x	x	✓	- Inconsistency and imprecision: adjustment for multiple testing essential
List of top DMCpGs/ DMRs including effect sizes & p-value	x	–	–	x	x	–	✓	- Inconsistency and imprecision: p-values and effect sizes are essential to judge the strength of association
Calculated proportion of DMCpGs or DMRs in the primary analysis at the stated level of significance as a proportion of all measured	x	x	x	x	x	x	✓	- Imprecision
Effect sizes	Low/very low	Large	Low/very low	Not provided	Low/very low	moderate*	Very low	- Inconsistency and imprecision: p-values and effect sizes are essential to judge the strength of association
Inflation measured	x	–	–	x	–	–	x	- Imprecision
Validation, replication or meta-analysis	✓ Replication attempted but not achieved	x	x	x	x	x	✓ Replication attempted in a subset of older children but not achieved	- Imprecision
Functional association of methylation differences	x	x	x	x	✓ Gene expression	✓ Protein levels	✓ Gene expression	- Inconsistency

(*Continued*)

Table 3. (*Continued*)

	Randomised controlled trial	Cohort studies						
Assessment criteria	ROLO study Geraghty *et al.* (2018)	C-MACH Miyaso *et al.* (2017)	NEST House *et al.* (2018)	'Feeding fetus' low-grade inflammation and insulin resistance study Bianchi *et al.* (2019)	Isle of Wight 3rd Generation Cohort Losol *et al.* (2019)	Fat intake study Chiu et al. (2021)	PACE consortium Küpers et al, 2022	Grade criteria
How were the results reported in the title/ abstract?	Results over-stated Authors stated: 'preliminary evidence' – incorrect due to failed replication	Accurately Authors stated: 'changed' which wrongly implies a longitudinal study	Accurately Authors did not mention the small effect sizes	Accurately Authors stated: 'potential to influence the offspring DNA methylation', 'validation . . . warranted'	Results over-stated Authors stated: 'Induced . . . DNA methylation' but no evidence of causation	Accurately Authors used: Cautious phrases e.g., 'suggest' and 'may influence'	Relatively accurately Authors did not mention the four statistical models	- Inconsistency and imprecision
Overall evaluation of quality of epigenetic analysis	Good Low sample size, unknown effect size	Good Using calorie intake as a continuous variable may have been better. Moderate sample size but large effect size	Good Moderate sample size but low effect size	Poor Using omega-3 PUFA intake as a continuous variable may have been better. Moderate sample size, unknown effect sizes	Poor No adjustment for multiple testing, insufficient details provided on array analysis & statistical model. Small sample size and low effect size	Poor No adjustment for multiple testing, some outcome variables not discussed; specific p-values not provided. Small sample size and unknown effect size	Good Meta-analysis of large sample size, stringent adjustment for multiple testing and covariates, very low effect size, no evidence of stability across time in children	Low certainty

Key: ✓, performed, ∇, not performed; -, not clear; n/a, not applicable due to study design. Sample size classification (per group): <100, small; 100–1,000, moderate; 1000+, large. Effect size classification: <1%, very low; 1%–5%, low; 5%–10%, moderate; >10% large on a scale of 0-100% difference).

*Denotes an effect size that is not directly comparable as it reflects a ratio rather than exposed vs non-exposed.

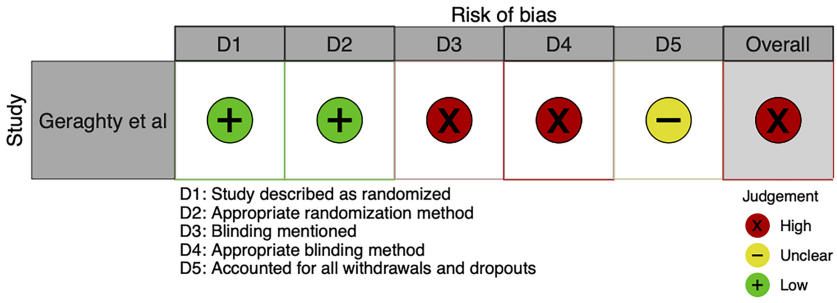

Figure 2. Jadad scale for reporting randomised controlled trials.

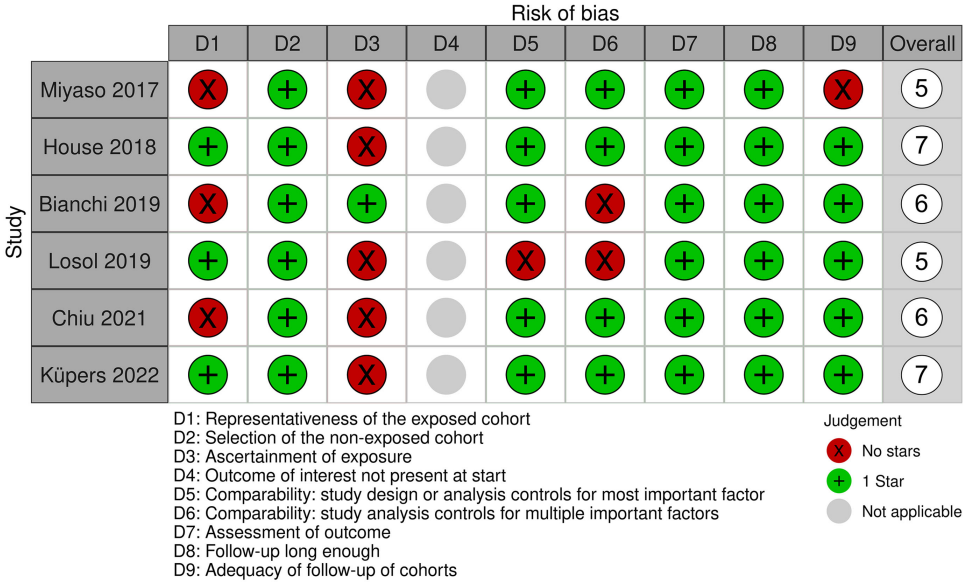

Figure 3. Newcastle-Ottawa assessment of bias in cohort studies. As infant epigenetic data are not available prenatally, all studies have 'not applicable' for D4 (outcome of interest not present at start).

this limits the representativeness of the sample (D1), the sample homogeneity may help to improve the precision of the results by limiting variation in DNA methylation due to other factors.[37] The 'Feeding fetus' study selected a non-representative subsample of the original population, excluding 84% of the sample (847/1000) due to smoking and health conditions.[35] Unlike Miyaso *et al.*,[45] this did not influence the scoring for D9 as Bianchi *et al.*[35] explicitly investigated a healthy population, rather than excluding due to missing records. Similarly, Isle of Wight 3rd generation cohort study[36] investigated a subset of the Isle of Wight cohort, however, they confirmed that the subcohort was representative of the larger cohort, hence scored one star for D1 and D9.

Accuracy of the dietary exposure measures

All studies scored one star for D2 (selection of the non-exposed cohort) as cohort studies dichotomised the exposure groups using an aspect of diet meaning that the non-exposed cohort selection was a subset of the selected cohort. All but the Feeding fetus study[35] scored no stars for D3 (ascertainment of exposure) as the dietary exposure measure was self-reported using an FFQ. Bianchi *et al.*[35] used a biomarker of dietary omega-3 PUFA exposure and scored one star for D3 being considered an objective 'secure record'. Chiu *et al.*[37] scored no stars for D3 as dietary omega-3 PUFA intakes were assessed using a validated FFQ including multivitamin supplement intakes during pregnancy. Although Chiu *et al.*[37] used previously validated FFQ-obtained estimates of omega-3 PUFA against erythrocyte and plasma fatty acids,[46,47] there were no biomarkers of omega-3 PUFA intake hence no stars were scored for D3.

Cohort comparability

Losol *et al.*[36] scored no stars for D5 (Comparability: study design or analysis controls for most important factor) and D6 (Comparability: study analysis controls for most important factors) for not controlling or adjusting analyses for important factors known to influence methylation. Strict inclusion/exclusion criteria were used in Bianchi *et al.*[35] to control for factors known to influence methylation, hence cohort comparability (D5) scored one star. The same study collected dietary intake using an FFQ; however, it was not used, and diet was not controlled for in the analyses hence (D6) scored no stars as unreported aspects of dietary intake may have biased the results.

Follow-up on study outcomes

Although epigenetic outcomes do not fit well with starred options for D7 (assessment of outcome) 'independent blind assessment' nor 'record linkage', they are of better quality than 'self-report' and 'not described'. The follow-up period was adequate for the outcome to occur in all studies scoring D8 (follow-up long enough).

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Table 4. GRADE certainty assessment of the evidence supporting the statement 'Specific dietary patterns in pregnancy are associated with epigenetic state in infants'

Outcomes	Number of studies, Number of participants	Range of effect	Study Limitations including risk of bias	Imprecision	Indirectness	Inconsistency	Publication bias	Quality of evidence (GRADE)
Methylation	7, 621	From low (<1%) to high (>10%)	Low certainty	Very low certainty	Low certainty	Low certainty	Low certainty	Low

Assessment of reporting and publication bias

Most of the studies did not explicitly state their primary and secondary outcomes, making it difficult to assess whether results were selectively reported or published. Bias in the selection of the reported results is possible as there was no evidence of data analysis pre-registration plans; however, most studies presented many null results not just favourable or significant results. The effect sizes reported in the included studies were small, indicating that selective non-reporting bias may only be minor, however, there still may be studies that were not published based on null results.

Certainty assessment

The results presented in this systematic review provide low certainty that specific dietary patterns in pregnancy are associated with epigenetic state in infants (Table 4). All studies had limitations and were assessed as having moderate to high risk of bias (Figs. 2 and 3). The certainty of the results was reduced by small sample sizes and the level of imprecision in the effect size estimates due to heterogeneous analysis methods (Table 3), including inconsistency in adjusting for confounders and adjusting for multiple testing. Not all studies directly addressed the research question 'how do specific prenatal dietary patterns associate with epigenetic state in infants?' as some only considered fish intake[36] or omega-3 PUFA intake[35–37] and excluded the rest of the diet from analysis or adjustment. Furthermore, the differences in the participant characteristics and baseline risk factors make it difficult to directly address this question (Table 1). There was inconsistency in the approaches used to measure and analyse DNA methylation data, contributing to the lower quality of evidence (Table 3). The certainty of these results is influenced by the potential for reporting or publication bias as studies may be missing. It is unclear whether these results overestimated the reported association. Therefore, the evidence presented in this systematic review provides low certainty that dietary patterns in pregnancy relate to epigenetic state in infants with very low to high effect sizes.

Discussion

This study is the first to systematically investigate the potential effect of maternal dietary patterns on infant epigenetic state in humans and encompasses seven studies published prior to April 2022. This review yielded low certainty, heterogeneous evidence that maternal dietary intake was associated with epigenetic state in infants with a range of effect sizes, from very low to large, across a range of sample sizes (from small to large). DNA methylation was analysed in cord blood neonatal dried blood spots or peripheral blood in mid-childhood (Saffari *et al.*, 2020) using either EWAS or gene-specific approaches. Low and very low magnitudes of effects are common in environmental epigenetic studies.[48–50] The effect sizes in our study ranged from very low (<1%), to low (1%–5%), moderate (5%–10%) and large (>10%). It is unclear how these effect sizes relate to health. However, one of the included studies found that adherence to a Mediterranean diet during pregnancy was associated with better behavioural outcomes, and sex-dependent differences in methylation at four infant DMRs.[51] Importantly, this study reported opposing differences in effects between the sexes for two DMRs (*SGCE/PET10*, *PLAGL1*). It is therefore difficult to translate this evidence, even into dietary recommendations as sex is not always known during pregnancy; moreover, further studies are needed to replicate this result. However, more broadly, the evidence is unequivocal that a healthier prenatal diet is consistently associated with better behavioural outcomes in children[52]; this may occur via epigenetic regulation, adequate nutrition, or may include other pathways such as the prenatal gut microbiota.[53] The health implications of differential methylation and sex-dependent or low effect sizes needs further investigation.[54] In the NEST study, there were sex-specific maternal MDA and associations of DNA methylation within *SGCE/PEG10*, *IGF2* and *MEG3* and child behaviours such as depression, anxiety, atypical and autism spectrum disorder-related behaviours.[51]

Folic acid has a well-established role in the methionine cycle, central to 1-carbon metabolism.[55] Unsurprisingly, epigenome-wide FDR-significant associations have been found between maternal plasma folate levels and infant DNA methylation (443 DMCpGs in 320 genes).[56] However, omega-3 PUFAs are also an important input to the methionine cycle,[53] which is needed for methylation reactions. In the present systematic review, three studies reported low to medium effect sizes for the association between maternal omega-3 PUFA intake and offspring DNA methylation.[35–37] One study used a biomarker to measure dietary intake,[35] which is favourable to a food frequency questionnaires or dietary recall due to lack of recall bias.[57] Another recorded the frequency of oily fish intake during trimester three, without defining a serve and did not measure full dietary intake.[36] It is unclear whether these results are confounded by unmeasured supplemental fish oil, as pregnant women frequently consume such supplements.[58] It is difficult to translate this evidence into concrete dietary recommendations as food frequency questionnaires and biomarkers do not capture full dietary intakes. Although theoretically possible, we were unable to conclude with certainty that prenatal dietary omega-3 PUFA intake relates to methylation in infants due to the small number of studies, different methods of measurement, small to moderate sample sizes and low methodological quality evidence.

Strengths and limitations

The strengths of this study include adherence to the PRISMA guidelines,[26,59] including rigorous assessment of study quality. The conclusions of this study were limited by study heterogeneity, including varied study quality, methods and differing exposures and outcomes. We caution drawing conclusions from dietary

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exposures that do not reflect full dietary intakes, or that only measure the maternal diet at one point in time. All of the included studies had deficits in dietary measurement. At best, the low glycaemic index study measured diet throughout pregnancy using an FFQ at every trimester. However, the participants were aware that their diet was reviewed by a dietitian[32] and this may have resulted in social-desirability bias.[60] Moreover, FFQs are inflexible and may not capture full dietary intake due to the limited number of food items surveyed.[61] FFQ can also suffer recall bias and problems estimating frequency and are therefore less accurate than 24-hour dietary recalls, or the gold-standard weighed food records.[61,62] Other studies measured diet in a manner that may miss changes in intakes. For example, the Isle of Wight 3rd Generation Cohort study recorded the frequency of oily fish consumed in the third trimester of pregnancy,[40] and the NEST study did not state the duration that the FFQ was recorded.[34] Eating habits during the recorded time may not completely reflect mothers' diet throughout pregnancy, due to food aversions and nausea.[63] The C-MACH study measured caloric intake.[33] However, this does not provide information on diet quality or the variety of foods consumed. Conversely, the NEST study[51] reflects a whole-of-diet approach that is more suitable for translation and adoption. The dietary exposure measures were heterogeneous across studies, making it difficult to draw concrete conclusions, or translate the evidence into dietary recommendations. There was heterogeneity in the ethnicities of the cohorts which may have limited precision. Different ethnicities could confound our results as diet quality[64] and genetic background can vary across ethnicities.[65] All participants recruited in the C-MACH study were from a Japanese population whereas participants in the other studies were mainly European in origin. This review provides low-certainty evidence that there is a relationship between maternal diet and epigenetic state of the infant. The lower certainty is due to limitations in study designs and analysis. Heterogeneity and quality DNA methylation analysis were evident across studies. This includes issues such as sample size; adjusting for multiple testing; testing and adjustment for technical, biological and environmental confounders; quality control of DNA methylation data, testing for genetic confounding of epigenetic state; testing for bias or inflation; and replication/meta-analysis. A major limitation in all seven studies was that other epigenetic mechanisms other than DNA methylation, such as histone modification and non-coding RNAs, were not examined. Therefore, changes to such states may have gone unnoticed.

Future recommendations

The limited number of studies in this field highlights the need for further high-quality work to evaluate the impact of maternal diet on infant epigenetic state. In designing these studies some of the following points should be considered. Total dietary intake should be measured while accounting for supplement use. Ideally, dietary intake should be recorded in a manner that reflects the maternal diet throughout pregnancy to enable dietary patterns to be derived, or calculation of diet quality indices using standardised scoring methods that indicate adherence to dietary guidelines (such as the Healthy Eating Index,[66] or a Mediterranean diet score).[67] Epigenetic modifications can be analysed in cord blood and other biosamples including buccal swabs/saliva and placenta, and we recommend that such analysis be considered alongside that of DNA methylation in future studies. This will provide a more complete picture of the epigenetic consequences of maternal nutrition in offspring, as alteration of each modification may not have the same effect on gene expression. The time-period for the maternal diet to have its effects on the offspring may vary; therefore, follow-up of children from birth cohorts is needed to ascertain the potential health relevance of methylation difference in infancy that may otherwise remain unnoticed in a study with a shorter time duration. Ideally, epigenetic state should be measured at a subsequent timepoint in childhood to determine whether epigenetic associations can last beyond birth. The parameters we used to assess quality of epigenetic analysis in this review were taken from personal experience of one author (JMC), from reviews on the analysis of environmental EWAS in children, and from EWAS methodology in general. We recommend that researchers familiarise themselves with the following issues at a minimum before planning their studies: sample size, tissue sampled, analytical platforms, confounders and covariates including cellular heterogeneity, quality control steps, genetic confounding and adjustment for multiple testing. We also recommend that researchers provide a list of DMCpGs/DMRs that includes raw and adjusted p-values and effect sizes and provide proportion of DMCpGs/DMRs at the stated level of significance should also be stated to enable comparison with other studies. We also recommend measuring inflation, which tests for the effects of technical and biological confounders. We recommend increasing study power by pooling data into meta-analyses via consortia and/or combining new data with previously published studies. Additionally, consulting with community stakeholders and pre-publishing study plans should be carried out more often. Finally, we encourage accurate reporting of results, avoiding erroneous conclusions about cause vs association and avoiding selective reporting of significant associations.

Conclusion

This study expands the previous reviews that have focused on individual dietary supplements[68–70] or micronutrients,[71–75] by investigating prenatal dietary intakes and epigenetic outcomes in infants. This review yielded low certainty, heterogeneous evidence that maternal dietary intake was associated with epigenetic state in infants with a range of effect sizes from very low to large across a range of sample sizes (from small to large). The conclusions of this study were limited by study heterogeneity, including varied study quality, methods and differing exposures and outcomes. Further, larger, well-designed studies are needed ideally with comprehensive dietary assessment and longer follow-up periods.

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