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Effects of dietary interventions on DNA methylation in adult humans: systematic review and meta-analysis

Khalil ElGendy[1,2]*, Fiona C. Malcomson[1], Jose G. Lara[3], David Michael Bradburn[2] and John C. Mathers[1]

[1]*Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne NE2 4HH, UK*
[2]*Surgery Department, Northumbria NHS Foundation Trust, Cramlington NE23 6NZ, UK*
[3]*Applied Sciences Department, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne NE1 8ST, UK*

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Abstract

DNA methylation is a key component of the epigenetic machinery that is responsible for regulating gene expression and, therefore, cell function. Patterns of DNA methylation change during development and ageing, differ between cell types, are altered in multiple diseases and can be modulated by dietary factors. However, evidence about the effects of dietary factors on DNA methylation patterns in humans is fragmentary. This study was initiated to collate evidence for causal links between dietary factors and changes in DNA methylation patterns. We carried out a systematic review of dietary intervention studies in adult humans using Medline, EMBASE and Scopus. Out of 22 149 screened titles, sixty intervention studies were included, of which 65 % were randomised (*n* 39). Most studies (53 %) reported data from blood analyses, whereas 27 % studied DNA methylation in colorectal mucosal biopsies. Folic acid was the most common intervention agent (33 %). There was great heterogeneity in the methods used for assessing DNA methylation and in the genomic loci investigated. Meta-analysis of the effect of folic acid on global DNA methylation revealed strong evidence that supplementation caused hypermethylation in colorectal mucosa ($P = 0{\cdot}009$). Meta-regression analysis showed that the dose of supplementary folic acid was the only identified factor ($P < 0{\cdot}001$) showing a positive relationship. In summary, there is limited evidence from intervention studies of effects of dietary factors, other than folic acid, on DNA methylation patterns in humans. In addition, the application of multiple different assays and investigations of different genomic loci makes it difficult to compare, or to combine, data across studies.

Key words: DNA methylation: Dietary interventions: Intervention studies: Systematic reviews: Meta-analysis and meta-regression

In humans, DNA is methylated by the addition of a methyl group to the 5′ position on cytosine (C) residues where the C is followed by a guanine (G) residue – that is, a CpG dinucleotide. This methylation is catalysed by DNA methyl transferase using *S*-adenosyl methionine (SAM) as the methyl donor. DNA methylation is a component of a suite of epigenetic marks and molecules, which also includes post-translational modification of histones and small non-coding RNA. These epigenetic mechanisms are functionally important because they are key players in the regulation of gene expression[1].

Patterns of DNA methylation change during development and ageing, differ between cell types and are altered in multiple diseases including cardiovascular and neoplastic diseases and neurological disorders[2]. Altered DNA methylation is an early and consistent event in the development of cancer, including colorectal cancer (CRC)[3], where it plays a causal role through silencing of tumour suppressor genes and activation of oncogenes. Aberrant DNA methylation patterns result in reduced DNA integrity and stability, development of mutations, changes in gene expression and chromosomal modifications[4]. DNA methylation, measured in target or surrogate tissues, has been developed as a diagnostic, prognostic or predictive biomarker for several diseases[5–7]. However, DNA methylation patterns differ between cell and tissue types and may respond differently to interventions[8] so that DNA methylation assayed in a surrogate tissue may not be reflective of the target tissue.

Patterns of DNA methylation respond to many environmental exposures and lifestyle factors including diet[1,9]. Nutritional factors can affect DNA methylation by modifying the activity of enzymes involved in DNA methylation such as DNA methyltransferase or by changing the availability of methyl donors for SAM synthesis[10]. Experimental studies using tissue culture and animal models have demonstrated effects of multiple dietary factors including polyphenols, flavonoids and phyto-oestrogens on DNA methylation[11], some of which have also been reported in observational studies in humans. However, folic acid supplementation remains the most widely studied nutritional factor affecting DNA methylation[12,13]. Most of the

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; RCT, randomised controlled trial.

* **Corresponding author:** K. ElGendy, email Khalil.elgendy@ncl.ac.uk

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evidence of effects of dietary factors on DNA methylation in humans comes from cross-sectional observational studies and there appear to be few relevant intervention studies[12].

The aim of this study was to undertake a systematic review of intervention studies in adult humans that involved diet or dietary factors and which reported DNA methylation as an outcome to (i) synthesise the evidence for causal links between specific dietary factors and corresponding changes in DNA methylation and (ii) ascertain the utility of easier-to-collect surrogate samples for investigating effects of dietary factors on DNA methylation in target tissues. To our knowledge, no prior systematic review has addressed these questions.

Methods

The systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist and flow chart[14] and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42017072315).

Search strategy and screening

A total of three databases were searched (Embase, Scopus and Medline) from inception until April 2017 by using the following search terms: ((methylation [Mesh] OR dna methylation [Mesh] OR methylat*) AND ((Supplement OR supplement* OR dietary supplements [Mesh]) OR (trial* OR clinical trial [Mesh]) OR (Intervention OR intervention*))).

Articles were screened against the following pre-specified inclusion criteria – (1) study design: any intervention study, randomised or non-randomised; (2) participants: adult human beings (≥16 years old); (3) intervention: dietary interventions (single, multiple or combined with other modalities – e.g. physical activity) and (4) outcome: DNA methylation measured using any methodology as an outcome (primary or secondary) assessed before and after the intervention. Where DNA methylation was assessed after the intervention only, randomised controlled trials (RCT) only were included in the review. Where DNA methylation was assessed before intervention only, studies were excluded.

Studies that recruited patients undergoing active treatment of cancer including chemotherapy or/and radiotherapy were excluded because of the likelihood that such therapies would confound the dietary effects. In studies involving pregnant women, the study was included if the outcome was assessed in tissue samples from the pregnant woman, but not if the measurements were made in the offspring or products of conception – for example, cord blood or placenta.

Titles and abstracts were screened independently by two independent investigators (K. E. and F. C. M.). This was followed by accessing full texts to ensure meeting inclusion and exclusion criteria. Any discrepancy regarding the decision to include a study was resolved by a third reviewer (J. C. M.).

Data extraction and quality assessment

The following data were collected using a pre-tested standard form: year of publication, study design, health or disease status of participants, number of participants, nature of dietary intervention, intervention duration, sample site, DNA methylation assessment method (including genomic loci, where appropriate), DNA methylation levels of participants before and after intervention with measures of variance and level of significance. These data were uploaded into Microsoft® Excel 2013 and used to compile a narrative synthesis of the results that is reported below using descriptive statistics (e.g. percentages) and summary tables.

Meta-analysis and meta-regression

Eligible studies were included in a meta-analysis conducted using the Review Manager software (version 5.3, the Cochrane Collaboration, 2014) and intervention effects were quantified using a random-effects model (owing to heterogeneity) and standardised mean difference (owing to different methods used to quantify DNA methylation). In addition, risk of bias was assessed using the Cochrane Collaboration's Risk of Bias tool. Heterogeneity between studies was assessed using χ^2 statistic (expressed as P value) and I^2 statistics (expressed as percentage) using Review Manager version 5.3.

Results of meta-analysis of different techniques for quantification of global DNA methylation (direct *v.* indirect measurement and different direction of effects) were examined using comprehensive meta-analysis (CMA) software (version 2; Biostat) using a random-effects model (owing to heterogeneity) and standardised mean difference. The CMA software was also used to carry out meta-regression analysis using a mixed-effect model, and publication bias was examined via funnel plots and Egger's regression test (expressed as P value).

Results

The PRISMA flow chart (online Supplementary Fig. S1) summarises the outcomes of the search strategy. Out of 22 149 titles, sixty intervention studies were included, of which thirty-nine studies (65 %) were RCT and seven (12 %) were cross-over RCT. The number of participants recruited per study ranged from 7 to 388, with a median value of 34.

Across sixty trials, twenty-two different dietary interventions were applied. The most common intervention agent was folic acid, which was tested in one-third of the studies (n 20, 33 %) followed by a low-energy diet (n 5, 8 %) and multivitamin supplements (n 5, 8 %) (online Supplementary Fig. S2). One-third of the studies (twenty trials) recruited healthy individuals, whereas participants with sixteen disease conditions or risk factors were studied in the remaining forty papers. Studies on patients with colorectal disease (n 13) represented 22 % of the total, whereas seven studies (11 %) recruited obese and/or overweight patients (online Supplementary Fig. S3).

A wide range of DNA methylation assessment methods (thirty in total; see online Supplementary Table S2) were used, and only ten studies (17 %) reported outcomes from a combination of types of DNA methylation assessment (online Supplementary Table S1). Global DNA methylation was investigated in more

than half of the trials (*n* 31, 52 %) and was the sole DNA methylation measurement in twenty-six studies (43 %). The most common techniques were the [3H]-methyl acceptance assay (*n* 9, 15 %) for estimation of global DNA methylation and Sequenom's MassARRAY EpiTyper (*n* 7, 12 %) for methylation at specific genomic loci. Bisulphite sequencing, using ten different techniques, was applied in more than one-third of the trials (*n* 21, 35 %) (online Supplementary Table S3).

Methylation in DNA extracted from six different tissues was studied (online Supplementary Fig. S4). Blood samples were used in more than half of the trials (*n* 32, 53 %), with leucocytes being the most common cell fraction studied (*n* 12, 20 %). Methylation in DNA from colorectal mucosal biopsies was reported in sixteen studies (27 %). Other tissues included adipose tissue, muscle, semen and mammary tissue. In the text below, the results of the intervention studies have been categorised according to the tissue/sample site and dietary intervention.

Effects of dietary intervention on DNA methylation in blood

Of the thirty-two trials that reported data from blood samples, seventeen were RCT with one cross-over RCT. In all, eight studies used folic acid as the intervention agent (Table 1)[15–22], whereas seven trials involved weight-loss interventions (Table 2)[23–29]. Other studies are summarised in Table 3[30–46].

Folic acid supplementation

Jacob *et al.*[15] and Rampersaud *et al.*[16] quantified global DNA methylation in postmenopausal females, and reported decreased methylation in response to folate depletion. Following folic acid supplementation, that change was revered in the study by Jacob *et al.*[15], but not in the study by Rampersaud *et al.*[16], who found no significant change after repletion in a study with greater power.

In male patients with hyperhomocysteinaemia, Ingrosso *et al.*[17] conducted a non-randomised folic acid supplementation study and observed significantly increased global DNA methylation, whereas Pizzolo *et al.*[18] reported no significant change after folic acid supplements in a non-RCT. Similarly, in an RCT involving 216 patients with hyperhomocysteinaemia, Jung *et al.*[22] found no effect of folic acid supplementation over 3 years on global DNA methylation in leucocytes. This lack of effect of folic acid supplementation on global DNA methylation was also observed in RCT involving healthy volunteers[20] and women of reproductive age[21].

The combination of folic acid with other nutrients involved in one-carbon metabolism including methionine[31], choline and betaine[39] and vitamin B_{12}[44] did not modify methylation at specific genomic loci (Table 3). An exception was Kok *et al.*[30] who investigated effects of folic acid (0·4 mg/d) and vitamin B_{12} (0·5 mg/d) and demonstrated significant changes in DNA methylation at many CpG sites in or close to *DIRAS3*, *ARMC8* and *NODAL* genes. (For full names of each of the genes listed in this paper by their ID, please see Supplementary Table S4.)

Weight-loss nutritional intervention

Nicoletti *et al.*[25] compared the effects of reduced dietary energy intake and bariatric surgery on DNA methylation in buffy coat samples from obese patients in a non-randomised study. Compared with baseline, methylation of *IL-6* increased in those exposed to dietary energy restriction and decreased in the bariatric surgery group. However, there was no effect of either intervention on global DNA methylation (assessed as methylation of the repeated element *LINE1*). Duggan *et al.*[29] did not detect any significant changes in *LINE1* methylation in leucocytes from 298 postmenopausal obese females after 1 year of exposure to an energy-restricted diet, exercise or both. Delgado-Cruzata *et al.*[28] reported that *LINE1* methylation increased after 6 months of a weight-loss programme involving both diet and exercise in twenty-four breast cancer survivors, whereas, in contrast, Martín-Núñez *et al.*[26] found significantly lower *LINE1* methylation in 310 participants after 9 months of intervention with a combination of Mediterranean diet, physical activity and education aiming at weight loss.

Effects of dietary intervention on DNA methylation in the colorectal mucosa

Methylation of DNA extracted from colorectal mucosal biopsies was investigated in sixteen studies, most of which (*n* 14) were RCT. The large majority (*n* 11) involved patients with colorectal adenomas, whereas only three studies recruited healthy participants. Other disease conditions included familial adenomatous polyposis and ulcerative colitis (one trial each). Folic acid was the intervention agent in ten trials (Table 4), whereas other intervention studies investigated effects of black raspberries, vegetables, non-digestible carbohydrates, *Bifidobacterium lactis*, high-amylose maize starch and combined folic acid and vitamin B_{12} (Table 5).

Effects of folic acid on DNA methylation status in colorectal biopsies differed between studies. In all, eight trials studied effects on global methylation. Figueiredo *et al.*[52] randomised 388 patients with adenoma to a folic acid supplement or a placebo and reported no effect on global DNA methylation. That finding was supported by results from another RCT[48] and from a non-RCT[53]. However, five RCT found increased global DNA methylation in adenoma patients following folic acid supplementation. Wallace *et al.*[54] and Al-Ghnaniem Abbadi *et al.*[55] found no effect of folic acid on DNA methylation of *SFRP1*, *ESR1* or *MLH1* in patients with adenoma. Findings from meta-analysis and meta-regression of the available evidence for the effects of folic acid supplementation on global DNA methylation are presented later in this article.

Wang *et al.*[60] found a significant lower methylation of *SFRP2* and *SFRP5* after consumption of black raspberries by patients at high risk of CRC, but there were no effects of this food on *LINE1*, *WIF1* or *SPRP2* methylation[61]. van den Donk *et al.*[57] reported significantly higher global DNA methylation and increased methylation of specific genes (*O6-MGMT*, *hMLH1*, *p14ARF*, *p16INK4A* and *RASSF1A*) but decreased *APC* methylation after use of folic acid and vitamin B_{12} supplements. Increased consumption of vegetables[58], non-digestible

Table 1. Effects of folic acid supplementation on DNA methylation in different blood samples

First author (year)	Design	*n*	Age (years)	Participants	Dose of folic acid	Duration	Blood product	Assessment method	Studied region or loci	Results
Jacob (1998)[15]	Non-RCT	8	49–63	Postmenopausal females (USA)	5 weeks of 56 μg/d, 4 weeks of 111 μg/d, 3 weeks of 286–516 μg/d	91 d	Lymphocyte	[3H]-methyl acceptance assay	Genome wide	↓ Methylation up to 111 μg/d reversed with repletion
Rampersaud (2000)[16]	Non-RCT	33	60–85	Postmenopausal females (USA)	Depletion for 7 weeks, then repletion with 200 or 400 μg/d	7 weeks	Leucocytes	[3H]-methyl acceptance assay	Global	No significant changes after repletion ↓ Methylation with 7-week depletion significantly
Ingrosso (2003)[17]	Non-RCT	43	61·3 (patients) 58·7 (controls)	Men with hyper-homocysteinaemia and uraemia with haemodialysis (Italy)	15 mg/d	8 weeks	PBMC	3H-cytosine extension assay	Global	↑ Methylation
Pizzolo (2011)[18]	Non-RCT	7	33–68	Hyper-homocysteinaemia MTHFR 677TT (Italy)	5 mg/d	8 weeks	Whole blood	Liquid chromatography–MS	Genome wide	No effect
Ellingrod (2015)[19]	Non-RCT	35	50 (SD 9)	Schizophrenia (70 % Caucasian – USA)	5 mg/d	3 months	Whole blood	Luminometric methylation assay	Global	↑ Methylation (especially with subjects on olazapine or colazpine)
Basten (2006)[20]	RCT	61	42 (intervention) and 40 (control)	Healthy volunteers (UK)	1·2 mg/d	12 weeks	Lymphocytes	[3H]-methyl acceptance assay	Global	No effect
Crider (2012)[21]	RCT	76	30 (SD 4)	Women of reproductive age (USA)	0·1 or 0·4 or 4 mg/d	6 months	Leucocytes	[3H]-methyl acceptance assay	Global	No effect Significance was observed in regard to coagulation of sample and genotype *MTHFR* CC *v.* TT
Jung (2011)[22]	RCT	216	60·9	Elevated homocysteine (Netherlands)	0·8 mg/d	3 years	Leucocytes	Liquid chromatography–MS	Global	No difference between placebo and treatment groups and groups stratified for *MTHFR* C677T

RCT, randomised controlled trial; ↓, decrease; PBMC, peripheral blood mononuclear cells; ↑, increase; *MTHFR*, methylenetetrahydrofolate reductase.

Table 2. Effects of weight-loss nutritional interventions on DNA methylation in different blood products*

First author (year)	Design	*n*	Age (years)	Participants	Intervention	Duration	Blood product	Assessment method	Studied region or loci	Results
Milagro (2011)[23]	Non-RCT	25	NA	Overweight or obese healthy men (Spain)	Restricted energy diet	8 weeks	PBMC	HumanMethylation27 BeadChip, Sequenom's MassARRAY EpiTyper	Genome wide, *ATP10A*, *WT1*, *CD44*, *IFNG*, *MEG3*, *TNFRSF9*, *AQP9*, *NTF3* and *POR*	↑ Methylation of *WT1* (CpG21) and *ATP10A* (CpG18)
Abete (2015)[24]	Non-RCT	40	64 (SD 1)	Ischaemic stroke with matched control (Spain)	Nutritional programme energy-restricted Mediterranean diet	20 weeks	Buffy coat	Sequenom's MassARRAY EpiTyper	*KCNQ1*, *WT1*	Ten CpG-*KCNQ1*: ↑ in stroke patients, ↓ in control Twenty-two CpG-*WT1*: ↓ in stroke patients
Nicoletti (2016)[25]	Non-RCT	45	31·7 (SD 8·6) (control), 52·6 (SD 9·9) (energy restriction) and 35·5 (SD 10·1) (surgery)	Obese patients (control, bariatric surgery: Brazil, energy restriction: Spain)	Control (normal healthy, *n* 9), energy restriction diet (RESMENA, *n* 22) and bariatric bypass surgery (*n* 14)	6 months for diet or follow-up after gastric bypass	Buffy coat	MethylFlash, EpiTect Fast	*LINE1*, *SERPINE1*, *IL6*	*IL6* methylation: ↑ after energy restriction and ↓ after bariatric surgery No change in *LINE1* or *SERPINE1*
Martín-Núñez (2014)[26]	Non-RCT	310	53·5 (control) and 54·6 (intervention)	Healthy volunteers (Spain)	Intervention programme (Mediterranean dietary pattern and exercise)	12 months	Whole blood	Pyromark Q96 ID	*LINE1*, *SCD1*	*LINE1*: ↑ in control ($P<0·001$) and ↓ with intervention ($P<0·004$) *SCD1*: ↑ in control ($P<0·001$)
Samblas (2016)[27]	Non-RCT	61	42·2 (SD 11·4)	Overweight or obese healthy women (Spain)	Weight loss programme (Mediterranean dietary pattern, physical activity, education, behavioural techniques)	9 months	Whole blood	Sequenom's MassARRAY EpiTyper	*BMAL1, NR1D1, CLOCK*	*BMAL1* (↑ 5, 6·7, 9, ↓ 10·11, 18) *NR1D1* (↑ 10, 17, 18, 22, ↓ 1, 19)
Delgado-Cruzata (2015)[28]	Cross-over RCT	24	52·2 (SD 8·7)	Hispanic, African American and Afro-Caribbean overweight female breast cancer survivor (USA)	Weight-loss programme (increased physical activity by 90/week, reducing energetic intake	6 months	Leucocytes	PyroMark Q24, LUMA, MethyLight	*LINE1*, *SAT2*	Significant ↑ in *LINE1* methylation
Duggan (2014)[29]	RCT	298	57·9 (SD 4·9)	Postmenopausal healthy overweight females 84·9 % are white (USA)	Reduced energy diet (*n* 82), exercise programme (*n* 70), both (*n* 87) *v.* control (*n* 59)	12 months	Leucocytes	PyromarkQ24	*LINE1*	No change

RCT, randomised controlled trial; NA, not available; PBMC, peripheral blood mononuclear cells; ↑, increase; CpG, cytosine-phosphate-guanosine; ↓, decrease; RESMENA, MEtabolic Syndrome REduction in Navarra; LUMA, luminometric methylation assay.

* For full names of each of the genes listed in this table by their ID, please see Supplementary Table S4.

Table 3. Effects of different dietary interventions (other than folic acid and weight-loss interventions) on DNA methylation in different blood products*

First author (year)	Design	*n*	Age (years)	Participants	Intervention	Duration	Blood product	Assessment method	Studied region or loci	Results
Kok (2015)[30]	RCT	87	70·8 (SD 2·9) (intervention) 71·1 (SD 3·0) (control)	Elderly (>65) with hyper-homo-cystenaemia (Netherlands)	Folic acid (0·4 mg/d) and vitamin B_{12} (0·5 mg/d) (*n* 44) *v.* placebo (*n* 43)	2 years	Buffy coat	HumanMethylation450 BeadChip	Genome wide	↑ Significantly in a single position (cg19380919) and six regions related to *DIRAS3*, *ARMC8* and *NODAL* genes
van der Kooi (2006)[31]	Non-RCT	15	43 (SD 16) (patients) 50 (SD 18) (controls)	Facio-scapulo-humeral muscular dystrophy (Netherlands)	Folic acid (5 mg/d) and methionine (1 g TDS)(*n* 9) Control (*n* 6)	12 weeks	Leucocytes	Phosphoimager	*D4Z4*	No effect
Shin (2010)[32]	Non-RCT	60	18–55	Folate compromised Mexican American men (USA)	Choline (300, 550, 1100 and 220 mg/d)	12 weeks	Leucocytes	Liquid chromatography–MS	Global	↓ in DNA methylation in *MTHFR* 677CC (more in 300 mg/d group) no change in *MTHFR* 677TT
Milenkovic (2014)[33]	Non-RCT	13	48, range 30–58	Non-obese, healthy male smokers (Netherlands)	200 mg monomeric and oligomeric flavanols from grape seeds	8 weeks	Leucocytes	HumanMethylation450 BeadChips	Genome wide	No changes Large inter-individual variability
Scoccianti (2011)[34]	RCT	88	51·19 (control), 53·65 (enriched diet) and 52·39 (supplemented diet)	Heavy smokers (Italy)	Isoenergetic diet, cruciferous veg, flavonoids (green tea, soya)	4 weeks	Leucocytes	PSQ 96MA	*LINE1*, *RASSF1A*, *ARF*, *CDKN2a*, *MLH1*, *MTHFR*	↑ *LINE1* No changes in other loci
Crescenti (2013)[35]	RCT	214	54·73 (control) and 59·75 (intervention)	Humans with CVD risk factors (pre-HTN, stage 1 HTN, hyper-hypercholesterolaemic) (Spain)	Cocoa (6 g/d, *n* 110) *v.* control (*n* 114)	2 weeks	Leucocytes	Agilent 1100 Series liquid chromatograph	Global	↓ Methylation No association with polymorphism of *DNMT*, *MTHFR* and *MTRR*
Greenlee (2016)[36]	RCT	70	56·6 (SD 9·7)	Hispanic breast cancer survivors (Columbia)	Culturally based 9-session programme to increase F/V intake and decrease fat (*n* 34) Control (*n* 36)	12 weeks	Leucocytes	PyroMark Q24	*LINE1*	↑ Methylation (P=0·06)
Zhu (2016)[37]	RCT	58	28·2 (placebo), 25·6 (600 IU/d; 15 μg/d), 24·7 (2000 IU/d; 50 μg/d) and 25·2 (4000 IU/d; 100 μg/d)	Vitamin D-deficient African American (USA)	Vitamin D_3 (600, 2000, 4000) *v.* placebo	16 weeks	Leucocytes	MethylFlash	Global	↑ Methylation in a dose-dependent manner
Apron (2017)[38]	RCT	36	64·6 (3·9) *v.* 63·5 (SD 1·7) *v.* 63·2 (SD 2·1)	Healthy with CVD risk factors (Spain)	Low-fat diet *v.* MedDiet/EVOO *v.* MedDiet/nuts	5 years	PBC	HumanMethylation450 BeadChip	*EEF2*, *COL18A1*, *IL4I1*, *LEPR*, *PLAGL1*, *IFRD1*, *MAPKAPK2*, *PPARGC1B*	Changes in all eight genes studied (no data for each individual group, no statistical data regarding significance)
Abratte (2009)[39]	RCT	45	24·2, range 18–46	Women of reproductive age (equal numbers of African Americans, Mexican, Caucasians, Asians, Arabs – USA)	Betaine, choline, folate (four groups, subgroup MTHFR C667T)	12 weeks	PBMC	3H deoxyCTP	Global	No effect
do Amaral (2014)[40]	RCT	12	35·1 (SD 5·5) (control) *v.* 23·4 (SD 5·0) (fish oil)	Overweight, under energy-restricted diet (Spain)	*n*-3-rich fish oil	8 weeks	PBMC	Sequenom's MassARRAY EpiTyper	*CD36*, *FFAR3*, *CD14*, *PDK4*, *FADS1*	Wight loss affected methylation especially at *CD36* gene (reduction), fish oil reduced the reduction in same gene in very small effect
Hoile (2014)[41]	RCT	29	53–63	Chronic renal failure (UK, Australia)	Olive oil or *n*-3 LCPUFA	8 weeks	PBMC	PSQ 96MA	5′ regulatory regions of *FADS2*, *FADS1*, *ELOVL5* and *ELOVL2*	Different effects and dependent on sex
Switzeny (2012)[42]	Non-RCT	15	66·30 (SD 5·89) *v.* 66·30 (SD 5·89)	Type 2 DM and IFG (Austria)	300 g of vegetables and 25 ml of plant oil	8 weeks	Whole blood	COBRA and PyroMark Q24	*MLH1*, *MSH2*, and *MGMT*	↑ in two *MLH1* promoter regions and *MGMT* promoter
Hubner (2013)[43]	Non-RCT	34	66·4 (SD 10·5)	Healthy adults (Germany)	Vitamin B, D, Ca (500 μg folic acid, 500 μg vitamin B_{12}, 50 mg vitamin B_6, 1200 IU (30 μg) vitamin D and 456 mg Ca)	1 year	Whole blood	PyrosequncingTM	*LINE1*	No effect (no difference between *MTHFR* subgroups)
Stopper (2008)[44]	RCT	27	60·3 (SD 8·6) (control), 64·4 (SD 10·9) (FA) and 68·2 (SD 16·4) (FA/B_{12})	Long-term haemodialysis (Germany)	Folic acid (5 mg three times weekly IV) ± vitamin B_{12} (1000 μg/week), control	20 weeks	Whole blood	Liquid chromatography–MS	Global	No effect
Hariri (2015)[45]	RCT	40	NA	Type 2 DM (Iran)	200 ml/d soya milk and 200 ml/d of probiotic soya milk containing *Lactobacillus plantarum* A7	8 weeks	Whole blood	Methylation-specific PCR-Q	*MLH1* and *MSH2*	↓ Methylation of *MLH1*, no effect on *MSH2*
Pusceddu (2016)[46]	RCT	60	68·25 (SD 10·12)	Elderly (Germany)	1200 IU (30 μg) vitamin D and 456 mg Ca ± vitamins B (500 μg folic acid, 500 μg B_{12}, 50 mg B_6)	12 months	Whole blood	PSQ 96 MA	*LINE1*	↓ Methylation (305 sites differed significantly between two groups)

RCT, randomised controlled trial; ↑, increase; TDS, *ter die sumendum* (three times per d); ↓, decrease; MTHFR, methylenetetrahydrofolate reductase; HTN, hypertension; F/V, fruits/vegetables; MedDiet, Mediterranean diet; EVOO, extra-virgin olive oil; PBC, peripheral blood cells; PBMC, peripheral blood mononuclear cells; LCPUFA, long-chain PUFA; DM, diabetes mellitus; IFG, impaired fasting glucose; NA, not available.

* For full names of each of the genes listed in this table by their ID, please see Supplementary Table S4.

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Table 4. Effects of folic acid supplementation on DNA methylation in colorectal mucosa*

First author (year)	Design	*n*	Age	Participants	Dose of folic acid	Duration	Sample	Assessment method	Studied region or loci	Results
Cravo (1994)[47]	RCT	32	63 (control) and 66 (FA)	Cancer, adenoma, healthy (Portugal)	10 mg/d	6 months	Rectum	[3H]-methyl acceptance assay	Global	↑ Methylation
Cravo (1995)[48]	RCT	20	56 (control) 39 (Crohns) 42 (UC)	Crohns and UC >7 years (Portugal)	5 mg/d	6 months	Colon	[3H]-methyl acceptance assay	Global	No effect
Cravo (1998)[49]	Cross-over RCT	20	57·6 (FA/placebo) and 55·7 (placebo/FA)	Adenoma (Portugal)	5 mg/d	3 months	Rectum	[3H]-methyl acceptance assay	Global	↑ Methylation
Kim (2001)[50]	RCT	20	62·2 (SD 3·2) (placebo) and 62·6 (SD 1·7) (FA)	Adenoma (USA)	5 mg/d	1 year	Rectum	[3H]-methyl acceptance assay	Global	↑ Methylation
Pufulete (2005)[51]	RCT	31	63·8 (placebo) and 63·9 (FA)	Adenoma (UK)	0·4 mg/d	10 weeks	Colon, leucocytes	[3H]-methyl acceptance assay	Global	↑ Methylation in both sample sites
Figueiredo (2009)[52]	RCT	388	57·8 (SD 9·1)	Adenoma (North America)	1 mg/d (with aspirin 81 mg or 325 mg) (3×2 factorial design)	3 years	Colon	PSQ HS 96 Pyrosequencing	*LINE1*	No effect
Protiva (2011)[53]	Non-RCT	20	54 (inpatient group) and 57·6 (outpatient group)	Healthy (60 % Caucasian, 30 % African American, mixed race, USA)	1 mg/d (with aspirin 81 mg or 325 mg) (3×2 factorial design)	8 weeks depletion then 4 weeks repletion	Rectum	Universal bead array system	Global	No effect
Wallace (2011)[54]	RCT	388	57·8 (SD 9·1)	Adenoma (North America)	1 mg/d (with aspirin 81 mg or 325 mg) (3×2 factorial design)	3 years	Colon	PyrosequncingTM	*ERa* and *SFRP1*	No effect
Al-Ghnaniem Abbadi (2013)[55]	RCT	29	63·2 (placebo) and 63·9 (FA)	Adenoma (UK)	0·4 mg/d	10 weeks	Rectum	PSQ HS 96 Pyrosequencing	*ESR1, MLH1*	No effect
O'Reilly (2016)[56]	RCT	20	68 (placebo) and 64 (FA)	Adenoma (Ireland)	0·6 mg/d	6 months	Colon	Modified alkaline comet assays	Global	↑ Methylation

RCT, randomised controlled trial; FA, folic acid; ↑, increase; UC, ulcerative colitis; *ERa*, oestrogen receptor α.
* For full names of each of the genes listed in this table by their ID, please see Supplementary Table S4.

Table 5. Effects of different dietary supplementation (other than folic acid) on DNA methylation in colorectal mucosa*

First author (year)	Design	*n*	Age (years)	Health status	Intervention	Duration	Sample	Assessment method	Studied region or loci	Results
van den Donk (2007)[57]	RCT	76	61·1 (intervention) 61·4 (placebo)	Adenoma and genotype *MTHFR 677* (Netherlands)	Folic acid (5 mg) and vitamin B_{12} (1·25 mg) *v.* placebo	6 months	Rectum	Methylation-specific PCR-Q	*APC*, *O6-MGMT* and *hMLH1*, *p14ARF*, *p16INK4A*, *RASSF1A*	↑ Methylation overall and all individual genes except *APC*
van Breda (2009)[58]	RCT	28	NA	Females with adenoma and healthy females (Netherlands)	Low (75 g/d) *v.* high (300 g/d) vegetable diet (carrots, cauliflower, peas, onions)	2 weeks	Rectum	UVI band-intensities quantification	*C-FOS*, *ODC1*, *MTHFR*, *PKCB1*	No effect
Worthley (2009)[59]	Cross-over RCT	20	60·4 (range 45–75)	Healthy adults (Australia)	High amylose maize starch (25 g/d) or *B. lactis* (5 g/d) or both	4 weeks for each, 12 weeks	Rectum	MethyLight, PSQ HS96 System	*LINE1*, *ESR1*, *GATA5*, *HIC1*, *HPP1*, *SFRP1*, *MLH1*, *CDKN2A*, *MINT1*, *MINT2*, *MINT31*, *CACNA1G*, *IGF2*, *RUNX3*, *NEUROG1*, *SOCS1* and *MGMT*	No effect
Wang (2011)[60]	Non-RCT	20	59	CRC, adenoma polyp (USA)	Black raspberries (oral) 60 g/d	1–9 weeks	Colon	MassARRAY, PSQ HS96	*Global*, *p16*, *PAX6a*, *SFRP2*, *SFRP5*, *WIF1*	↓ Methylation of *SFRP2* and *SFRP5*
Wang (2014)[61]	RCT	14	48 (range 30–67)	FAP (USA)	Black raspberries (oral and enema) 60 g/d *v.* placebo and enema	9 months	Rectum	MBDCap-seq, Pyromark, MassARRAY	*LINE1*, *p16*, *SFRP2*, *WIF1*	↓ Methylation of *p16* No effect on *LINE1*, or *SFRP2*, *WIF1*
Malcomson (2017)[62]	RCT	75	52·4 (range 30–80)	Healthy (97% Caucasian, UK)	Non-digestible carbohydrates (RS 23 g/d Hi-maize 260, polydextrose 12 g/d)	50 d	Rectum	Pyromark Q96 ID	*SFRP1*	No effect

RCT, randomised controlled trial; *MTHFR*, methylenetetrahydrofolate reductase; ↑, increase; NA, not available; UVI, UV imager; CRC, colorectal cancer; ↓, decrease; FAP, familial adenomatous polyposis; MDBC-seq, methyl-CpG binding domain-based capture and sequencing; RS, resistant starch.

* For full names of each of the genes listed in this table by their ID, please see Supplementary Table S4.

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Table 6. Effects of dietary interventions on DNA methylation in adipose cells*

First author (year)	Design	*n*	Age (years)	Participants	Intervention	Duration	Assessment method	Studied region or loci	Results
Bouchard (2010)[63]	Non-RCT	14	57·7 (low responders) and 57·8 (high responders)	Overweight and obese postmenopausal women (Canada)	Energy-restricted diet	6 months	CpG-island 15K microarray, Sequenom's MassARRAY EpiTyper	Genome wide	1p36, 4q21 and 5q13 loci were differentially methylated after intervention
Cordero (2011)[64]	Non-RCT	27	32–50	Obese women (Spain)	Energy-restricted diet	8 weeks	Methylation-specific PCR	*LEP*, *TNF-α*	No change
Gillberg (2014)[65]	Cross-over RCT	45	23–27	LBW (*n* 19), NBW (*n* 26) (Denmark)	Fat overfeeding *v.* control diet	5 d	Epigenetic sequencing methylation	*PPARGC1A*	↑ Methylation of *PPARGC1A* in LBW not NBW
Hjort (2017)[66]	Non-RCT	39	24·8 (LBW) and 24·6 (NBW)	NBW/LBW young adults (Denmark)	36-h fasting after 2 d of standard diet	36 h	Sequenom's MassARRAY EpiTyper	*LEP*, *ADIPOQ*	↑ Methylation only in NBW levels were higher in LBW than NBW
Perfilyev (2017)[67]	RCT	31	26·94 (SD 4·68) (SFA) and 27 (SD 4) (PUFA)	Healthy adults with BMI 18–27 kg/m^2 (Sweden)	SFA (muffins, 3 % weight gain, 51 % fat, 44 % CHO) (*n* 17) PUFA (refined sunflower oil) (*n* 14)	7 weeks	HumanMethylation450 BeadChips	Genome wide	Both diets increased mean methylation SFA ↑ methylation of 125 genes PUFA changed methylation of 1797 genes

RCT, randomised controlled trial; CpG, cytosine-phosphate-guanosine; LBW, low birth weight; NBW, normal birth weight; ↑, increase; *LEP*, leptin; *ADIPOQ*, adiponectin, C1Q and collagen domain containing; CHO, carbohydrates.
* For full names of each of the genes listed in this table by their ID, please see Supplementary Table S4.

carbohydrates[62] or maize starch/*B. lactis*[59] did not affect methylation of the specific genes studied in each of those trials (Table 5).

Effects of dietary interventions on DNA methylation in adipose tissue

Adipose tissue samples were obtained from subcutaneous tissues of the abdomen in four out of five intervention studies that investigated the effects of dietary interventions on DNA methylation (Table 6) (Cordero *et al.*[64] did not report the site of biopsy). In all, two non-RCT investigated the effect of energy restriction in obese women. Bouchard *et al.*[63] reported that energy restriction for 6 months altered methylation at three specific loci (*1p36*, *4q21* and *5q13*), whereas 8 weeks of restricted energy intake had no effect on methylation of *LEP* and *TNFα* in females of reproductive age[64].

Hjort *et al.*[66] found that 36 h fasting after 2 d of a standard diet increased methylation of *LEP* and *ADIPOQ* significantly in normal-birth-weight (NBW) adults but not in those with low birth weight (LBW). In contrast, Gillberg *et al.*[65] reported that overfeeding with fat increased methylation of *PPARGC1A* in adults with LBW but not those of NBW. Overfeeding with a diet rich in saturated and unsaturated fatty acids increased mean genome-wide methylation (assayed using a BeadChip Array; Illumina) in healthy adults[68].

Effects of dietary interventions DNA methylation in other tissues

Table 7 summarises findings from studies that reported effects of dietary interventions on DNA methylation in muscle biopsies, mammary cells and semen. In all, three cross-over RCT studied effects of high-fat overfeeding on DNA methylation on muscle cells of *vastus lateralis* in healthy adults, and one study[69] reported that this intervention increased *PPARGC1A* methylation in NBW adults.

DNA methylation in mammary cells was investigated in two RCT[67,72], with no significant change observed after interventions with soya isoflavones or with *trans*-resveratrol. However, Zhu *et al.*[67] found a significant inverse correlation between methylation of *RASSF1A* and serum *trans*-resveratrol concentration in healthy women at increased risk of breast cancer.

Methylation of DNA in semen after folic acid supplementations was assessed in two intervention studies[73,74]. Folic acid supplements resulted in reduced global DNA methylation in men with idiopathic infertility[73] but had no effect on global DNA methylation in healthy fertile men[74].

Meta-analysis and meta-regression of effects of folic acid supplementation on global DNA methylation

A total of five RCT used the [3H]-methyl acceptance assay for quantification of global DNA methylation in colorectal mucosal samples. In all, one study[48] was excluded as the study reported the significance of results only following folic acid supplementation but did not provide numerical data on DNA

Table 7. Effects of dietary interventions on DNA methylation in specialised tissues (mammary tissue, muscle cells and semen)*

First author (year)	Design	*n*	Age (years)	Participants	Intervention	Duration	Assessment method	Studied region or loci	Results
Muscle biopsy (*vastus lateralis*)									
Brons (2010)[69]	Cross-over RCT	46	24·6 (NBW) and 24·2 (LBW)	NBW *v.* LBW young adults (Denmark)	High-fat overfeeding (50 % extra energy with 60 % fat) *v.* control	5 d (6 weeks washout)	Epigenetic sequencing methylation	*PPARGC1A*, *NDUFB6*	↑ Methylation of NBW *NDUFB6* no change
Jacobsen (2012)[70]	Cross-over RCT	21	24·6 (SD 1·1)	Healthy men (Denmark)	High-fat overfeeding (50 % extra energy with 60 % fat) *v.* control	5 d (6 weeks washout)	Illumina's Infinium Bead Array (27 K)	Genome wide	Variable changes Delay to reverse changes
Jacobsen (2014)[71]	Cross-over RCT	40	24·6 (NBW) and 24·1 (LBW)	NBW *v.* LBW young adults (Denmark)	High-fat overfeeding (50 % extra energy with 60 % fat) *v.* control	5 d (6 weeks washout)	Illumina's Infinium Bead Array (27 K)	Genome wide	Larger changes observed in NBW No significant difference between two groups
Mammary cells									
Qin (2009)[72]	RCT	34	37 *v.* 36	Healthy premenopausal women (USA)	Soya isoflavones (40 mg/d) *v.* (140 mg/d)	10 d	Methylation-specific PCR-Q	*p16*, *RASSF1A*, *RARβ2*, *ER*, *CCND2*	No change
Zhu (2012)[67]	RCT	30	NA	Healthy adult women with increased risk of breast cancer (USA)	*trans*-Resveratrol (50 mg *v.* 5 mg) *v.* placebo	12 weeks	Methylation-specific PCR-Q	*p16*, *RASSF1A*, *APC*, *CCND2*	No significant effect ↓ Methylation of *RASSF1A* with ↑ serum *trans*-resveratrol levels
Semen specimen									
Aarabi (2015)[73]	Non-RCT	30	37·9 (SD 1·3)	Men with idiopathic infertility (Canada)	Folic acid (5 mg/d)	6 months	PyroMark Q24 kit, RRBS	*Global*, *H19*, *DLK1*, *GTL2*, *MEST*, *SNRPN*, *PLAGL1*, *KCNQ1OT1*	↓ Global methylation (more in MTHFR homozygous) No change on specific loci
Chan (2017)[74]	RCT	19	33 (SD 2) (placebo) and 36 (SD 2) (supplement)	Men with no infertility (Canada)	Folic acid (0·4 mg/d) (*n* 10) *v.* placebo (*n* 9)	3 months	RLGS assays, MCIp and array hybridisation, HumanMethylation450 BeadChip, MassArray Epityper	Genome wide	No effect

RCT, randomised controlled trial; NBW, normal birth weight; LBW, low-birth-weight; ↑, increase; ER, oestrogen receptor; NA, not available; ↓, decrease; RRBS, reduced representation bisulfite sequencing; MTHFR, methylenetetrahydrofolate reductase; RLGS, restriction landmark genomic scanning; MCIp, methyl-CpG immunoprecipitation.

* For full names of each of the genes listed in this table by their ID, please see Supplementary Table S4.

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methylation. For the remaining four studies, meta-analysis showed that folic acid supplementation increased global DNA methylation significantly ($P<0{\cdot}0001$) but there was significant heterogeneity between the included trials (I^2: 91 %, $P<0{\cdot}001$). Overall, there was low or unclear risk of bias owing to failure of reporting of randomisation and blinding (Fig. 1).

Meta-regression was used to investigate the effects of dose and duration of folic acid supplementation. This revealed that the dose of folic acid had a highly significant ($P=0{\cdot}0046$) and positive effect on global DNA methylation (online Supplementary Fig. S5), whereas there was no detectable effect of the duration of intervention ($P=0{\cdot}41$).

Considering different techniques of quantification of DNA methylation, eight RCT were included for meta-analysis with two subgroups: colorectal (*n* 6) and blood samples (*n* 3, as Pufulete *et al.*[51] reported data for both colorectal and blood samples). Folic acid increased DNA methylation overall ($P=0{\cdot}048$) and in colorectal mucosal samples specifically ($P=0{\cdot}002$) (Fig. 2). However, there was no significant effect of folic acid on DNA methylation in blood samples ($P=0{\cdot}468$). There was significant heterogeneity in the data for the colorectal subgroup ($I^2=91\,\%$, $P\leq0{\cdot}001$), blood subgroup ($I^2=84\,\%$, $P=0{\cdot}002$) and overall ($I^2=89\,\%$, $P<0{\cdot}001$). The test for subgroup differences was also significant ($P=0{\cdot}04$, $I^2=75{\cdot}6\,\%$) (online Supplementary Fig. S6). No high risk of bias was identified, but information to assess risk of bias was limited owing to incomplete reporting of randomisation, allocation concealment and blinding (online Supplementary Fig. S6).

Meta-regression analysis showed that, when investigated across both tissues and all analytical methods, the dose of folic acid used for supplementation had a highly significant and positive effect on global DNA methylation ($P=0{\cdot}0003$, Fig. 3). However, it should be noted that this effect is driven by changes in the colorectal mucosa as there was no evidence for an effect on DNA methylation in blood (online Supplementary Fig. S7). Duration of folic acid supplementation ($P=0{\cdot}35$) and post-intervention concentration of folate in serum (0·69) had no significant effect.

Assessment of publication bias

Investigation of potential publication bias was performed by producing a forest plot (Fig. 4) and statistical analysis using Egger's test ($P=0{\cdot}03$), and this revealed a risk of publication bias for Cravo *et al.*[47]. This study recruited patients with a history of either adenoma or carcinoma, whereas other studies recruited participants with a history of adenoma only. As a sensitivity analysis, meta-analysis was performed with inclusion of results of global DNA methylation in colorectal mucosal samples from the adenoma group only[47]. There was no change in risk of publication bias or significance of results (colorectal subgroup: $P=0{\cdot}02$, overall effect: $P=0{\cdot}04$, and Egger's test for publication bias: $P=0{\cdot}0025$, online Supplementary Fig. S7). Re-analysis of the data after exclusion of Cravo *et al.*[47] (online Supplementary Fig. S8) revealed a positive trend towards global DNA hypermethylation with folic acid supplementation in both the colorectal subgroup ($P=0{\cdot}08$) and overall ($P=0{\cdot}22$).

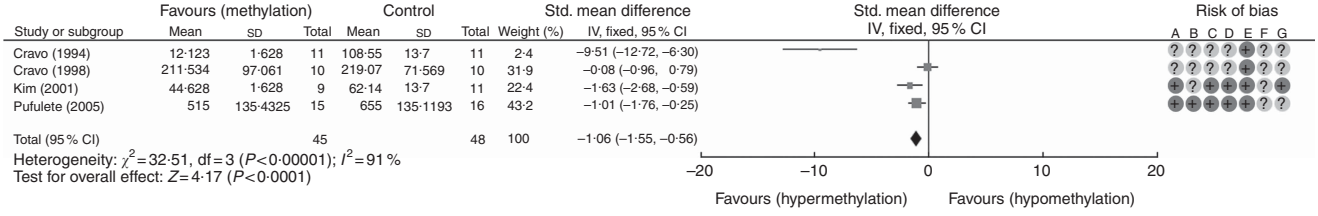

Fig. 1. Forest plot and risk of bias assessment of randomised controlled trial studying the effects of folic acid supplements on global DNA methylation in colorectal mucosal samples using [3H]-methyl acceptance assay using Review Manager (version 5.3).

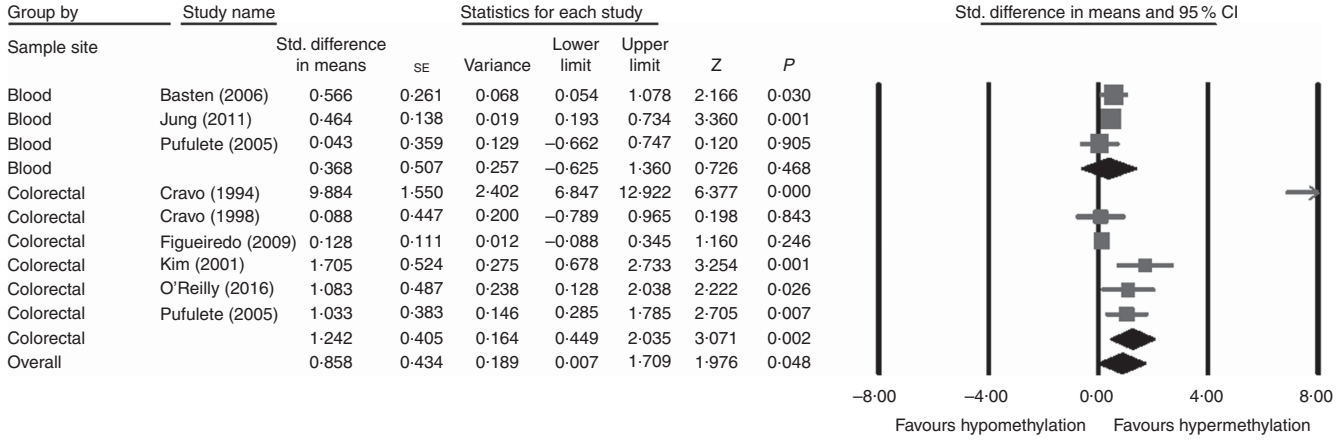

Fig. 2. Forest plot of randomised controlled trial studying effects of folic acid supplements on global DNA methylation in colorectal and blood samples using different techniques of quantification of DNA methylation using CMA software (version 2).

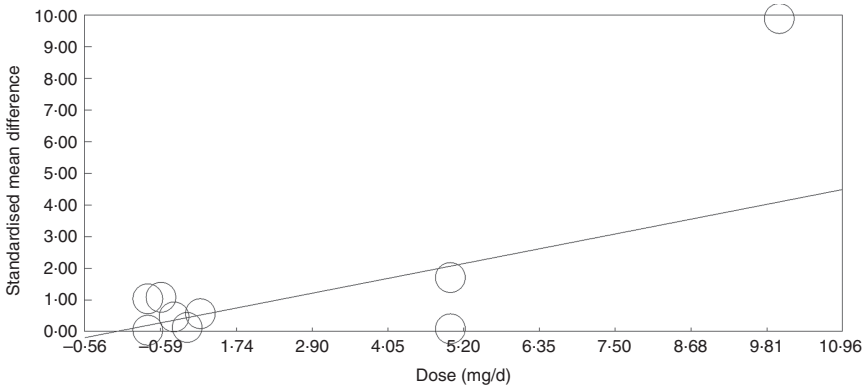

Fig. 3. Meta-regression of standardised mean difference in relation to the dose of folic acid supplements in the eight randomised controlled trial that were included in meta-analysis involving different techniques of quantification of DNA methylation.

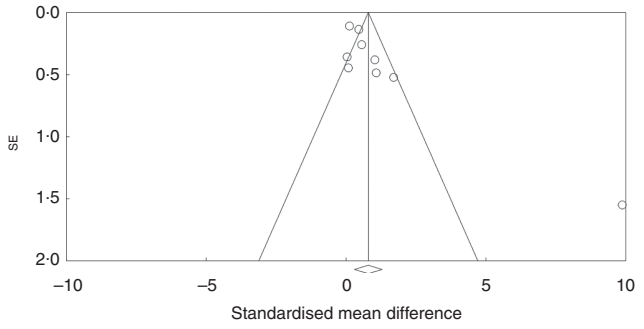

Fig. 4. Funnel plot of standard error by standard differences in means of the eight randomised controlled trial included in the meta-analysis.

Stratification according to methylenetetrahydrofolate reductase genotype

A total of eight intervention studies stratified patients according to a polymorphism in the gene coding methylenetetrahydrofolate reductase (*MTHFR*; 677C→T variant). When stratification according to *MTHFR* genotype was applied, a significant change in DNA methylation was reported in four studies. Crider *et al.*[21] reported a significant increase in global DNA methylation of leucocytes in those participants with the TT variant only following folic acid supplementation, whereas depletion of folic acid caused a significant decrease in global DNA methylation in carriers of the CC variant only. On the other hand, Aarabi *et al.*[73] reported that folic acid supplementation decreased DNA methylation in semen in participants with the TT variant. Global DNA methylation in leucocytes decreased following supplementation with choline in carriers of the CC variant[32] and following cocoa supplementation in those with the TT variant[35].

Discussion

Principal findings

We identified, and analysed data from, sixty dietary intervention studies in adult human participants that reported effects on DNA methylation. Most studies (53 %) reported data from blood analyses, whereas 27 % studied DNA methylation in colorectal mucosal biopsies. Some studies investigated effects on global DNA methylation, which were assessed using both direct and indirect methods. The methyl acceptance assay was the assay used most frequently for this purpose, but several studies also assessed methylation of the repeat element *LINE1*, which makes up 17–18 % of the human genome and which has been shown to be an acceptable surrogate for global DNA methylation in many cases[75]. Other studies interrogated specific genomic loci using either targeted – for example, Sequenom's MassARRAY EpiTyper – or genome-wide – for example, Illumina Bead array – approaches. Folic acid was the most common intervention agent (33 %) followed by low-energy diet (8 %) and multivitamins (8 %). Meta-analysis revealed that folic acid supplementation increased global DNA methylation significantly in colorectal mucosal samples, whereas meta-regression analysis showed that the dose of supplementary folic acid was the only significant factor ($P<0{\cdot}001$) causing this positive relationship.

In all, four out of eight intervention studies reported significant changes in DNA methylation following folic acid supplementation when participants were stratified according to *MTHFR* 677C→T genotype. Carriage of the T variant at position 677 in the of *MTHFR* gene is associated with lower folate status, higher circulating homocysteine concentration, reduced global DNA methylation and with increased risk of many disorders[76,77], including greater cancer risk[78,79]. This finding highlights the importance of considering subgroup classification according to *MTHFR* polymorphism in future research in effects of folic acid supplementation.

In all, two[15,16] out of three non-RCT reported a significant effect of folate depletion in decreasing global DNA methylation in blood products, but this effect was not observed in colorectal samples[53]. While Jacob *et al.*[15] observed that folic acid repletion reversed the DNA hypomethylation, no such effect was apparent in the study by Rampersaud *et al.*[16]. The participants in the latter study were older (>63 years) than those studied by Jacob *et al.*[15] (49–63 years), and it is possible that age blunted the speed of response to nutritional repletion. In this systematic review, there was no detectable effect of folic acid supplementation on DNA methylation in blood, but there was a significant effect on methylation of DNA from colorectal mucosal samples.

Possible mechanisms responsible for these findings

The mechanism responsible for such tissue differences in response to folic acid supplementation is not known. In human intervention studies, folic acid supplementation raises folate concentrations in both blood and the colorectal mucosa[80] so that it seems unlikely that there would be differential availability of methyl groups for synthesis of SAM for DNA methylation within blood cells and colonocytes. However, studies in mice have shown that folate depletion leads to tissue-specific effects on DNA methylation at selected genomic loci[8]. In addition, reduced circulating concentration of folate in blood was associated with DNA hypomethylation in human diabetic liver[81]. Such observations are consistent with cell-type-specific differences in cellular distribution of available methyl groups and/or differences in policing of the DNA methylome.

Strengths and limitations

Poor diet and diet-related factors are major contributors to the burden of ill health, especially cancer and cardiometabolic diseases[82]. This review summarises the available evidence for the impact of dietary factors on DNA methylation in both health and disease. Our systematic review shows that, in humans, little is known about the effects of dietary interventions on DNA methylation in tissues other blood and colorectal mucosa; only one-fifth of the included intervention studies in this review investigated other tissues. In addition, none of the included RCT correlated DNA methylation levels between target tissues and other surrogate tissues. The availability of validated assays for DNA methylation biomarkers in reliable and accessible surrogate tissues, such as blood, would avoid the need for invasive sample collection procedures, such as colorectal biopsies, which would facilitate larger population-based studies[83].

This systematic review faced many challenges in data summary and synthesising the evidence. The effects of dietary interventions on DNA methylation are gene and site specific, dependent on cell type and target tissue, and dose and duration of the interventions[4]. There was great heterogeneity in the methods used for assessing DNA methylation and in the genomic loci investigated. Samples were collected from both healthy individuals and from people with specific diseases, which contributed to the heterogeneity in the available data. Statistical heterogeneity was observed in the meta-analysis of eight trials, which had all tested effects of the same nutrient (folic acid). Most of the included RCT failed to report randomisation methods, allocation concealment or blinding that could lead to selective bias owing to poor choice of methods[84] and could affect outcome assessment[85]. Failure to report such important methodological aspect results in the inability to assess the risk of bias, which could compromise the overall strength of evidence.

Conclusion

Folic acid supplementation increases global DNA methylation in the colorectal mucosa in a dose-dependent manner. This observation may provide the basis for future research in prevention of bowel cancer as DNA hypomethylation is a consistent event in colonic carcinogenesis[3]. However, little is known about the effects of other dietary factors on DNA methylation patterns in any human tissue. In addition, multiple assays and different genomic loci have been used in investigations of effect of dietary interventions on DNA methylation, which makes it difficult to compare or combine data across studies. Standardisation of outcome measurements would facilitate future research.

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K. E. contributed to formulating the research question, designing the study, carrying the study out, analysing the data and writing the manuscript. F. C. M. contributed as the second independent screener of the titles, and reviewed the manuscript. J. G. L. assisted in designing the study and reviewed the manuscript. D. M. B. was involved in critical review of the manuscript and final approval. J. C. M. was involved in formulating the research question, designing the study, writing up and critical review of the manuscript, as well as in final approval.

The authors declare that there are no conflicts of interest.

Supplementary materials

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S000711451800243X

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