Journal of Nutrition & Intermediary Metabolism 18 (2019) 100104

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

journal homepage: www.elsevier.com/locate/jnim

Folate and Inflammation – links between folate and features of inflammatory conditions

Patrice Jones[a,b,*], Mark Lucock[a], Christopher J. Scarlett[a], Martin Veysey[a,c], Emma L. Beckett[a,b]

[a] School of Environmental & Life Sciences, University of Newcastle, Ourimbah, NSW, Australia
[b] Public Health Program, Hunter Medical Research Institute, New Lambton Heights, NSW, Australia
[c] Hull York Medical School, University of Hull, Hull, UK

HIGHLIGHTS

- Optimal folate levels may prevent endothelial dysfunction in inflammatory diseases.
- Folate may also alter inflammatory responses via DNA methylation and synthesis processes.
- The link between folate and inflammation varies based on several factors, such as timing of intervention.
- Further studies are needed before making folate intake recommendations around inflammation.

ARTICLE INFO

Keywords:
Folate
Inflammation
Endothelial dysfunction
DNA methylation
DNA synthesis

ABSTRACT

Folate serves as a cofactor for one-carbon (1C) transfer reactions. These reactions are involved in the synthesis of DNA nucleotides, the amino acid methionine, and in the regulation of homocysteine (Hcy) levels. Emerging evidence suggests that these reactions have roles in the development and maintenance of inflammatory responses, with optimal folate availability having key importance in preventing endothelial dysfunction and DNA instability. Low folate levels are commonly observed in chronic inflammatory diseases, indicating that inadequate folate may be involved in the pathogenesis of inflammatory conditions or that chronic inflammation increases folate requirements. These findings highlight folate interventions as a potential treatment in inflammatory disorders. However, current understanding of folate and its influence on inflammatory phenotypes is limited. Evidence indicates that the relationship between folate and inflammation is dependent on several factors, including the timing of intervention, dosage, and interaction with environment and genes. These factors require further investigation before recommendations for folate intake can be made for the prevention and treatment of inflammation. This review outlines the emerging role of folate in inflammation and key factors that may influence this relationship.

1. Introduction

Folate is a B vitamin derived from natural sources, such as green leafy vegetables, or from fortified foods or supplements. Folate-related roles within the human body are reliant on reduced folate compounds (i.e., tetrahydrofolates (THF)) holding and donating one-carbon (IC) units for 1C transfer reactions. These reactions are required in the synthesis of DNA nucleotides and the amino acid methionine, and the subsequent regulation of homocysteine (Hcy) levels [1].

Folate-dependent 1C reactions have been extensively studied in the context of cardiovascular disease and cancers, with increased risk of these diseases in those with inadequate folate status [1]. In addition to these well-established roles, interest in folate as a contributory factor in inflammation is emerging. This interest follows studies demonstrating cardiovascular-related outcomes and chronic inflammatory diseases share characteristics which may relate to folate status, such as endothelial dysfunction [2,3]. Endothelial dysfunction is defined as an impairment in vasomotor tone regulation via imbalanced levels of vasodilator and vasoconstrictors, which results in the endothelium shifting towards a pro-inflammatory state [4,5]. Folate may prevent endothelium dysfunction by maintaining levels of Hcy and vasodilator, nitric oxide (NO) [4–6]. In addition, a link between chronic inflammatory conditions such as inflammatory bowel diseases and elevated risk of carcinomas is well-established, and likely involves the role

* Corresponding author. School of Environmental & Life Sciences, University of Newcastle, Ourimbah, NSW, Australia.
E-mail address: Patrice.Jones@uon.edu.au (P. Jones).

https://doi.org/10.1016/j.jnim.2019.100104
Received 29 June 2019; Received in revised form 31 October 2019; Accepted 6 November 2019
Available online 09 November 2019
2352-3859/ © 2019 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

of folate in regulating DNA stability via mechanisms related to DNA methylation, synthesis and repair [7,8].

Observational evidence suggests a relationship exists between folate levels and risk of inflammatory disease. Low serum folate and elevated Hcy levels are commonly reported in patients with chronic inflammatory disorders, such as inflammatory bowel disease and rheumatoid arthritis [9,10]. However, results of *in vivo* and *in vitro* studies examining folate supplementation in inflammation have been inconsistent. Interpretation of these studies is further complicated by several factors that may alter the influence folate has in inflammation, such as the timing of intervention, folate dosage, and interaction with environmental and genetic factors. These factors require further consideration before recommendations for folate intake can be made for the prevention and/or treatment of inflammation.

This review outlines emerging roles of folate in inflammation, encompassing regulation of endothelial dysfunction, via both Hcy-dependent and Hcy-independent mechanisms, and the maintenance of DNA stability through DNA methylation, synthesis and repair reactions. Key factors that may modulate the activity of folate in these processes are also discussed.

2. Regulation of Hcy

Hcy is a non-essential amino acid produced as an intermediate product in the synthesis of essential amino acids methionine and cysteine. At elevated levels, Hcy can cause endothelial dysfunction, resulting in a shift towards a pro-inflammatory state [4].

Folate may modulate Hcy-induced endothelial dysfunction through its role as a major regulator of Hcy. Folate, in the form of 5-methylTHF, is used in the remethylation of Hcy to methionine (Fig. 1). This process involves the irreversible action of 5,10-methylenetetrahydrofolate reductase (MTHFR) in reducing 5,10-methyleneTHF to 5-methylTHF, and then B_{12}-dependent methionine synthase utilising 5-methylTHF as a methyl donor in the remethylation of homocysteine to methionine [11,12].

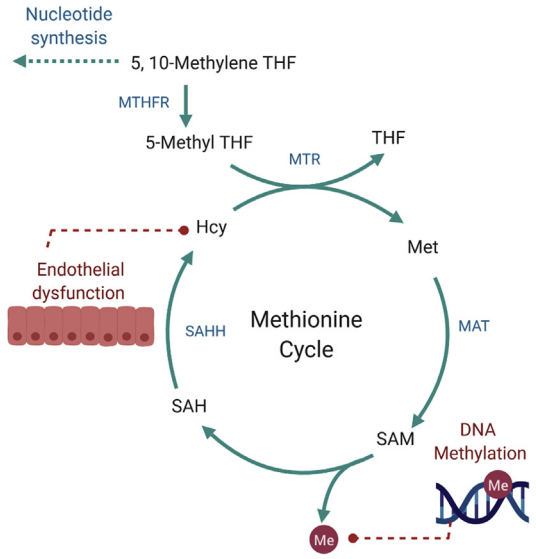

Fig. 1. Folate-dependent remethylation of Hcy to methionine.
The remethylation of Hcy involves the irreversible action of MTHFR in reducing 5,10-methyleneTHF to 5-methylTHF to be used by B_{12}-dependent MTR in the remethylation of Hcy to methionine. This process has dual importance in regulating Hcy and methionine levels. Hcy at elevated levels may cause endothelial dysfunction while methionine is the substrate for SAM, a universal methyl donor used in DNA methylation.
Abbreviations; THF; tetrahydrofolate, MTHFR; 5,10methyleneTHF reductase, MTR; methionine synthase, Hcy; homocysteine, Met; methionine, SAM; S-adenosyl methionine, MAT; adenosylmethionine synthetase, SAH; S-adenosyl hcy, SAHH; S-adenosylhomocysteine hydrolase.
Created with BioRender.

Hcy-induced endothelial dysfunction has been extensively studied in atherosclerosis but is increasingly considered in other inflammatory disorders, such as diabetes (types 1 and 2) and inflammatory bowel disease, which also features endothelium dysfunction [2,3]. The major mechanism by which Hcy induces endothelial dysfunction is through decreasing production of the vasodilator NO, which may occur via several mechanisms [4,5,13–19].

NO is the major vasodilator within the endothelium and is produced from L-arginine via endothelial nitric oxide synthase (eNOS). The activity of eNOS may be hindered by actions of Hcy in increasing levels of reactive oxygen species (ROS) and decreasing levels of eNOS cofactor, tetrahydrobiopterin (BH4). Hcy can cause ROS production through auto-oxidation of its sulfhydryl group [15] or through upregulating ROS-generating NADPH oxidases [5,13,14]. Increased ROS production leads to decreased bioavailability of BH4, which is readily oxidised to 7, 8-dihydrobiopterin (BH2) in the circulation [4,16]. Decreased BH4 bioavailability can further increase oxidative stress, where, in the absence of BH4, eNOS uncoupling occurs and eNOS produces the superoxide radical instead of nitric oxide [17].

Hcy may impact NO production through several other routes, however these are less well-characterised. Endothelial cells supplemented *in vitro* with Hcy (0.5–2.5 nM) showed dose-dependent decreases in the expression of L-arginine transporters [18], suggesting that Hcy may also influence NO production via regulation of L-arginine cell uptake. Hcy may also regulate eNOS activity by influencing levels of eNOS inhibitor, asymmetric dimethyl arginine (ADMA) [19]. In an *in vitro* study, elevated Hcy caused increased ADMA levels and decreased NO production via suppression of ADMA inhibitor dimethylarginine dimethylaminohydrolase [19]. Impaired NO production is the major mechanism by which Hcy may cause endothelial dysfunction, with further roles of elevated Hcy levels in decreasing hydrogen sulphide production and antioxidant activity, and increasing lipid peroxidation also influencing factors [4,5].

3. Hcy-independent regulation of NO & ROS production

Folate has actions in regulating NO & ROS production that are independent of roles in regulating Hcy levels. These actions are not traditional 1C transfer reactions but arise due to structural similarities between folates and biopterin compounds (i.e., between BH4 and THF, and BH2 and DHF) [6,20–23].

As outlined, eNOS cofactor BH4 is readily oxidised to BH2 in the circulation. Folate-dependent dihydrofolate reductase (DHFR) has a role in reducing BH2 back to BH4 via the BH4 salvage pathway (Fig. 2) [20,21]. This pathway has importance in maintaining an adequate BH4:BH2 ratio, with DHFR inhibition shown to reduce BH4 and increase BH2 levels *in vitro*, leading to enzymatic uncoupling of eNOS and increased ROS production [20,21].

Due to structural similarities between folates and biopterins, in the same way, BH2 may interact with DHFR, folate compounds are found to interact with eNOS [22]. 5-methylTHF is shown to bind to the active site of eNOS [22], and mimic the BH4's action in inducing NO production and reducing ROS production [22,24]. Through these mechanisms, folate supplementation (50 μM) has been found to increase NO production in an *in vitro* study examining human pulmonary artery endothelial cells under hypoxia [25]. This finding has been replicated in human subjects, with local perfusion of folate (5 mM of 5-MTHF) into the dermal space, and folate supplementation (6 mg/day) over 6 weeks both shown to improve in NO-dependent vasodilation and endothelial function in healthy older adults [25,26].

In addition to the roles of folate in regulating ROS production indirectly via prevention of eNOS uncoupling, folate may reduce oxidative stress by acting directly as an antioxidant [27,28]. Folic acid and reduced folate forms (THF and 5-MTHF) have shown ROS scavenging

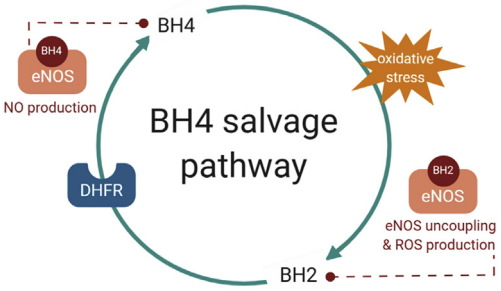

Fig. 2. The BH4 salvage pathway.
eNOS cofactor BH4 is readily oxidised to BH2 in circulation. In cases of decreased BH4 bioavailability, eNOS uncoupling occurs, and eNOS produces ROS instead of NO. BH2 may be reduced back to BH4 via folate-dependent DHFR via the BH4 salvage pathway. This pathway maintains an adequate BH4: BH2 ratio to support NO production.
Abbreviations; eNOS; endothelial nitric oxide synthetase, BH4; tetrahydrobiopterin, BH2; 7, 8-dihydrobiopterin, DHFR; dihydrofolate reductase.
Figure created with BioRender.

activity *in vitro* [27,28]. However, whether this activity has biological relevance remains under question, with it difficult to examine the direct and indirect effects of folate on ROS production separately in *in vivo* studies [6].

4. Maintaining DNA methylation

Methionine is produced through folate-dependent Hcy remethylation and is the substrate for S-adenosyl methionine (SAM), a universal methyl donor used in methyl transferase processes such as DNA methylation (Fig. 1) [12,29]. Through roles in influencing SAM levels, folate may modulate the bidirectional relationship between DNA methylation and inflammation, where hypomethylation of inflammation-related genes may predispose inflammatory diseases [30–32], and inflammation may promote DNA methylation processes [33–35]. DNA methylation changes are most dynamic during development and as such, a consistent research interest has centred on the potential impact dietary folate may have *in utero* on DNA methylation and future disease risk [36]. Of importance to inflammation, are several animal studies that demonstrate maternal folate intake may influence the risk of obesity and colitis in offspring [37–39].

Obesity is a low-grade inflammatory disorder and a further risk factor for chronic inflammatory diseases [40]. A relationship is evident between maternal weight gain and increased weight in children [41,42], with these findings replicated in several murine models [30–32]. Offspring of dams fed high fat obesogenic diets during pregnancy and lactation showed increased adiposity as adults. These offspring also possess increased insulin resistance and levels of pro-inflammatory cytokines [30,31,43], with this shown to increase susceptibility to colitis in one model [43]. In a murine model, supplementation of maternal diets with methyl donors (15 mg folic acid and 15 g choline/betaine/kg diet) ameliorated the effect of a high fat maternal diet on weight in offspring [37]. A further study in sheep found the maternal restriction of methyl donors resulted in heavier adult offspring that possessed altered immune responses and insulin resistance [38]. In a murine model of colitis, the offspring of dams fed methyl-donor deficient diets had more severe colitis and overexpression of pro-inflammatory pathways [39]. These findings indicate that folate status can influence the relationship between DNA methylation and inflammatory phenotype.

Inflammation is shown to induce DNA methylation changes in a multitude of *in vitro* and *in vivo* studies [44–50]. In response to inflammation, the expression of DNA methyltransferases (DNMTs), S-adenosylmethionine synthetase, and S-adenosylhomocysteine hydrolase (SAHH) involved in regulating DNA methylation and methionine production is increased [33–35]. This results in substantial increases in DNA methylation that may exacerbate inflammatory responses and promote tumorigenesis [44–50]. Increases in folate have been shown to be associated with increases in DNMT expression in human umbilical vein endothelial cells [51], with an increase in pro-inflammatory mediators reported in an murine model of folate restriction [52]. These findings indicate inflammatory responses may be enhanced in cases of folate deficiency [51,52]. In a murine model of colitis, inhibition of cellular methylation via SAHH inhibition lead to disease exacerbation, with intraperitoneal injections of folate acid (50 mg/kg) ameliorating colitis severity via controlled DNA methylation promotion [34]. This finding demonstrates that both hypomethylation and hypermethylation may cause pro-inflammatory effects, and that folate is an important factor balancing these effects.

5. Maintaining DNA synthesis & repair

Folate has a multifaceted role in nucleotide synthesis, with actions in synthesising the thymine precursor, thymidylate, and purines. In thymidylate synthesis, cytosolic serine hydroxymethyl transferase (SHMT), thymidylate synthase (TYMS) and DHFR use 5,10-methyleneTHF in the methylation of deoxyuridine monophosphate to form thymidylate monophosphate. Folate in the form of 10-formylTHF is also utilised in the synthesis of purine ring structures (Fig. 3) [12]. Nucleotide production is increasingly important during prolonged inflammation as it allows for DNA repair and the proliferation of immune cells.

Increases in ROS during inflammation can cause DNA mutations which may further contribute to inflammation and can lead to tumorigenesis [53]. Folate-dependent thymidylate is particularly important in DNA repair, with involved enzymes, SHMT, TYMS and DHFR, forming a multienzyme-complex that translocates to the nucleus during times of DNA synthesis and repair [54,55]. In a randomised clinical trial examining arsenic-exposed individuals (n = 450), supplementation with either 0.4 or 0.8 mg/day of folic acid for 8 weeks resulted in a significant decrease in oxidative DNA damage [56]. In comparison, *in vitro* and *in vivo* depletion studies both show folate deficiency to exacerbate DNA damage via reductions in thymine causing uracil to be misincorporated in DNA, and DNA repair responses to be hindered [57–60].

Folate deficiency is shown to reduce proliferation in multiple cell types by causing nucleotide imbalances and subsequent accumulation of cells in the S phase [61–64]. Notably, changes in folate levels may influence the proliferation of T cells [64], with aberrant T-cell proliferation associated with autoimmune chronic inflammatory diseases such as rheumatoid arthritis [65]. In cultured lymphocytes, folate depletion reduced the proliferation of $CD8^+$ T lymphocytes and increased the $CD4^+$ to $CD8^+$ T-cell ratio [64]. Supplementation of folate (300 nM) to folate-depleted lymphocytes decreased the $CD4^+$ to $CD8^+$ ratio [64]. $CD4^+$ T (helper/inducer) and $CD8^+$ (cytotoxic/suppressor) represent two T-cell subtypes, with an increased $CD4^+$ to $CD8^+$ ratio indicative of increased immunodeficiency, and a hallmark of autoimmune inflammatory diseases [65]. Further mouse model studies support a role of folate in maintaining levels of CD4 + Foxp3 regulatory T cells (Tregs) [66,67], a regulatory $CD4^+$ subtype dysregulated in chronic inflammatory diseases such as inflammatory bowel disease [68]. Folate receptors have been found to be highly expressed in natural Treg cells in mice, with blockage of these receptors resulting in decreased Treg levels [66,67].

6. Factors influencing inflammatory roles of folate

With emerging roles in maintaining inflammatory-related pathways, the potential usefulness of folate supplementation in the prevention and treatment of inflammation is being explored. However, data from

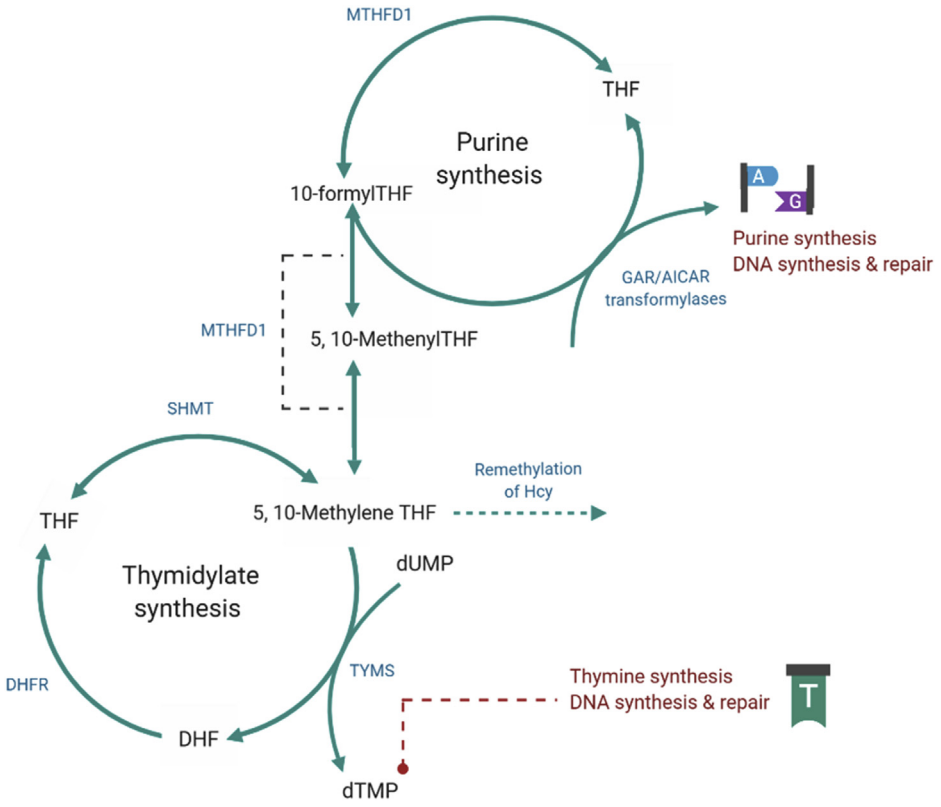

Fig. 3. Roles of folate in nucleotide synthesis.
Folate synthesises thymine precursor, thymidylate, and purine ring structures. In thymidylate synthesis, SHMT generates 5,10methyleneTHF which is then used by TYMS in methylation of dUMP to dTMP. DHFR regenerates oxidised DHF back to THF. Folate in the form of 10-formylTHF is utilised in the synthesis of purines, where GAR and AICAR transformylases transfer one carbon units from 10-formylTHF to purine ring structures. MTHFD1 has action in generating the different THF forms of folate supporting these reactions, as well as reactions in Hcy remethylation.
Abbreviations; THF; tetrahydrofolate, MTHFD1; methylenetetrahydrofolate dehydrogenase 1, GAR Glycinamide ribonucleotide; AICAR 5-aminoimidazole-4-carboxamide ribonucleotide; SHMT; serine hydroxymethyl transferase, DHFR; dihydrofolate reductase, TYMS; thymidylate synthase, dUMP; deoxyuridine monophosphate, dTMP; deoxythymidine monophosphate, Hcy; homocysteine.
Created with BioRender.

animal and human studies examining the influence of folate supplementation on inflammatory biomarkers is inconsistent and varies significantly based on factors such as timing of intervention (i.e., preventative vs. treatment interventions), folate dose, levels of other dietary factors, and genetic variance in folate enzymes.

Several human intervention studies demonstrate folate supplementation effectively lowers Hcy levels but this has varied influence on markers of endothelial dysfunction depending on time of intervention [69–75]. Hcy-lowering had no effect on markers of endothelial dysfunction in studies examining healthy subjects or subjects with current inflammatory conditions (2–5 mg mg/day of folic acid, 6 weeks–2 years) [69–72] but did improve endothelial function in subjects with elevated Hcy (5–10 mg/day folic acid over 6–8 weeks) [74,75]. These findings indicate folate supplementation is a more effective preventative measure rather than a potential treatment, particularly in individuals with elevated Hcy at risk of future inflammatory conditions.

Folate interventions for prolonged periods or in advanced disease stages may cause folate to promote inflammation by encouraging aberrant methylation and cell proliferation activity. Intraperitoneal injections of folate acid (50 mg/kg) ameliorated colitis severity in one murine model of colitis [34] but in further murine models supplementation of methyl donors to maternal diets (5 mg/kg folic acid, 5 g betaine and 5.76 g choline/kg diet) was found to induce susceptibility to colitis in offspring [76,77]. Supplementation of methyl donors to maternal diets (15 mg folic acid and 15 g choline/betaine/kg diet) was shown to combat the effect of a high fat maternal diets on weight gain in offspring in one model [37], but the opposite effect was seen in cases of excess folate supplementation (20 g folic acid/kg diet) [78]. This suggests level of folate dose may also largely determine the influence folate may have in inflammation.

As methyl donor supplementation is not a targeted approach, affecting methylation of both anti-inflammatory and pro-inflammatory genes, it is possible that prolonged or excessive exposure to methyl donors or intervention at specific disease stage may promote DNA methylation patterns that contribute to inflammation [79]. Prolonged or excessive exposure to folate during advanced diseases stages may exacerbate inflammation by also encouraging aberrant cell proliferation of pro-inflammatory and cancerous cell types [80]. Notably, methotrexate is an anti-folate medication with action in deterring proliferation of pro-inflammatory cells by inhibition of the role of folate in DNA synthesis. Methotrexate (MTX) is commonly prescribed in rheumatoid arthritis and other inflammatory disorders [81]. However, increased Hcy levels and risk of cardiovascular disease is commonly reported in methotrexate users [82] with current consensus that users should regularly use folate supplementation to offset these risks (recommended 5 mg folic acid following MTX treatments) [81].

The interpretation of current findings is made difficult by the coexistence of several dietary and genetic factors that regulate folate or folate-related processes. These factors are not always considered in study designs but are important nutrient-nutrient and nutrient-gene interactions that may determine study outcomes. In addition to folate, adequate levels of vitamin B_{12} and vitamin B_6, and methyl donor choline are required for Hcy-regulation, DNA methylation, and DNA synthesis [12,83]. Consequently, results of interventions can vary depending on whether folate supplementation was considered in isolation or in combination with relevant B vitamins and methyl donors. The occurrence of functional polymorphisms in folate-dependent enzymes may also influence the actions of folate in inflammation. *MTHFR*-C677T is the most extensively studied polymorphism in folate metabolism and is associated with significant reductions in MTHFR activity and folate status [84,85]. Results of *in vivo* and *in vitro* studies indicate *MTHFR*-C677T as a major genetic determinant of Hcy levels [86–89], with this variant also linked to changes in NO production [90], DNA methylation [91,92] and DNA damage [93,94] through interaction with folate status. Other functional polymorphisms in rate-limiting enzymes such as TYMS and DHFR are associated with changes in folate levels [85] and could theoretically alter inflammatory roles, but are yet to be examined. Considering the current available evidence, further consideration of factors such as timing of intervention, folate dose and interactions with dietary and genetic factors is needed in future studies

to elucidate the roles of folate in inflammation.

7. Conclusion

There is a growing body of evidence supporting the role of optimal folate availability in the development and maintenance of processes linked to inflammation. Folate may prevent endothelial dysfunction by Hcy-dependent and Hcy-independent regulation of NO levels. Additionally, actions of folate in DNA methylation, repair and synthesis processes may influence the inflammatory phenotype via epigenetic changes and modulation of cell proliferation. However, much of our current knowledge in how folate may influence inflammation is based on findings from *in vitro* studies and murine models, which have not been yet been translated into investigations of human cohorts. The interpretation of current findings is further complicated by several confounders that may alter the influence of folate, which need to be further examined before folate recommendations around inflammation can be made.

Author statement

Conception and design of review by PJ and EB. Drafting, revision, and approval of final submission by all authors.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Patrice Jones is supported by an Australian Government Research Training Program scholarship and a Hunter Medical Research Institute (HMRI) Greaves Family Scholarship. Emma Beckett is supported by a NHMRC Early Career Fellowship.

References

[1] P.J. Stover, Physiology of folate and vitamin B12 in health and disease, Nutr. Rev. 62 (6 Pt 2) (2004) S3–S12 discussion S13.
[2] C.M. Steyers, F.J. Miller, Endothelial dysfunction in chronic inflammatory diseases, Int. J. Mol. Sci. 15 (7) (2014) 11324–11349.
[3] A.S. De Vriese, et al., Endothelial dysfunction in diabetes, Br. J. Pharmacol. 130 (5) (2000) 963–974.
[4] W.K.C. Lai, M.Y. Kan, Homocysteine-induced endothelial dysfunction, Ann. Nutr. Metabol. 67 (1) (2015) 1–12.
[5] R. Esse, et al., The contribution of homocysteine metabolism disruption to endothelial dysfunction: state-of-the-art, Int. J. Mol. Sci. 20 (4) (2019) 867.
[6] A.E. Stanhewicz, W.L. Kenney, Role of folic acid in nitric oxide bioavailability and vascular endothelial function, Nutr. Rev. 75 (1) (2017) 61–70.
[7] C.N. Bernstein, et al., Cancer risk in patients with inflammatory bowel disease: a population-based study, Cancer 91 (4) (2001) 854–862.
[8] S. Danese, A. Mantovani, Inflammatory bowel disease and intestinal cancer: a paradigm of the Yin-Yang interplay between inflammation and cancer, Oncogene 29 (23) (2010) 3313–3323.
[9] Y. Pan, et al., Associations between folate and vitamin B12 levels and inflammatory bowel disease: a meta-analysis, Nutrients 9 (4) (2017) 382.
[10] P.E. Lazzerini, et al., Hyperhomocysteinemia, inflammation and autoimmunity, Autoimmun. Rev. 6 (7) (2007) 503–509.
[11] H.J. Blom, Y. Smulders, Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects, J. Inherit. Metab. Dis. 34 (1) (2011) 75–81.
[12] P.J. Stover, M.S. Field, Trafficking of intracellular folates, Adv. Nutr. Int. Rev. J. 2 (4) (2011) 325–331.
[13] K. Bedard, K.-H. Krause, The NOX Family of ROS-generating NADPH oxidases: Physiology and pathophysiology, Physiol. Rev. 87 (1) (2007) 245–313.
[14] S.H. Chan, et al., Exercise intervention attenuates hyperhomocysteinemia-induced aortic endothelial oxidative injury by regulating SIRT1 through mitigating NADPH oxidase/LOX-1 signaling, Redox Biol. 14 (2018) 116–125.
[15] I.F.W. McDowell, D. Lang, Homocysteine and endothelial dysfunction: a link with cardiovascular disease, J. Nutr. 130 (2) (2000) 369S-372S.
[16] N.J. Alp, K.M. Channon, Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease, Arterioscler. Thromb. Vasc. Biol. 24 (3) (2004) 413–420.
[17] G. Topal, et al., Homocysteine induces oxidative stress by uncoupling of NO synthase activity through reduction of tetrahydrobiopterin. Free radical biology & medicine, 36 (12) (2004) 1532–1541.
[18] L. Jin, et al., Homocysteine induces endothelial dysfunction via inhibition of arginine transport, J. Physiol. Pharmacol. 58 (2) (2007) 191–206.
[19] L.H. Liu, et al., Protection of DDAH2 overexpression against homocysteine-induced impairments of DDAH/ADMA/NOS/NO pathway in endothelial cells, Cell. Physiol. Biochem. 30 (6) (2012) 1413–1422.
[20] M.J. Crabtree, A.B. Hale, K.M. Channon, Dihydrofolate reductase protects endothelial nitric oxide synthase from uncoupling in tetrahydrobiopterin deficiency, Free Radic. Biol. Med. 50 (11) (2011) 1639–1646.
[21] M.J. Crabtree, et al., Critical role for tetrahydrobiopterin recycling by dihydrofolate reductase in regulation of endothelial nitric-oxide synthase coupling: relative importance of the de novo biopterin synthesis versus salvage pathways, J. Biol. Chem. 284 (41) (2009) 28128–28136.
[22] M.E. Hyndman, et al., Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function, Am. J. Physiol. Heart Circ. Physiol. (6) (2002) 282 p. H2167-72.
[23] S. Kaufman, Some metabolic relationships between biopterin and folate: implications for the “methyl trap hypothesis”, Neurochem. Res. 16 (9) (1991) 1031–1036.
[24] E.S. Stroes, et al., Folic acid reverts dysfunction of endothelial nitric oxide synthase, Circ. Res. 86 (11) (2000) 1129–1134.
[25] K. Chalupsky, et al., Folic acid promotes recycling of tetrahydrobiopterin and protects against hypoxia-induced pulmonary hypertension by recoupling endothelial nitric oxide synthase, Antioxidants Redox Signal. 23 (14) (2015) 1076–1091.
[26] A.E. Stanhewicz, L.M. Alexander, W.L. Kenney, Folic acid supplementation improves microvascular function in older adults through nitric oxide-dependent mechanisms, Clin. Sci. (Lond.) 129 (2) (2015) 159–167.
[27] R. Joshi, et al., Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity, Free Radic. Biol. Med. 30 (12) (2001) 1390–1399.
[28] B.M. Rezk, et al., Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore, FEBS (Fed. Eur. Biochem. Soc.) Lett. 555 (3) (2003) 601–605.
[29] L.M. Stead, et al., Methylation demand and homocysteine metabolism, Adv. Enzym. Regul. 44 (1) (2004) 321–333.
[30] G.J. Howie, et al., Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet, J. Physiol. 587 (Pt 4) (2009) 905–915.
[31] N.G. Ashino, et al., Maternal high-fat feeding through pregnancy and lactation predisposes mouse offspring to molecular insulin resistance and fatty liver, J. Nutr. Biochem. 23 (4) (2012) 341–348.
[32] Z. Vucetic, et al., Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes, Endocrinology 151 (10) (2010) 4756–4764.
[33] H.M. O'Hagan, et al., Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands, Cancer Cell 20 (5) (2011) 606–619.
[34] D.J. Kominsky, et al., An endogenously anti-inflammatory role for methylation in mucosal inflammation identified through metabolite profiling, J. Immunol. 186 (11) (2011) 6505–6514.
[35] E. Foran, et al., Upregulation of DNA methyltransferase–mediated gene silencing, anchorage-independent growth, and Migration of colon cancer Cells by interleukin-6, Mol. Cancer Res. 8 (4) (2010) 471–481.
[36] R. Barouki, et al., Developmental origins of non-communicable disease: implications for research and public health, Environ. Health 11 (2012) 42.
[37] J. Carlin, R. George, T.M. Reyes, Methyl donor supplementation blocks the adverse effects of maternal high fat diet on offspring physiology, PLoS One 8 (5) (2013) e63549.
[38] K.D. Sinclair, et al., DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status, Proc. Natl. Acad. Sci. 104 (49) (2007) 19351–19356.
[39] M. Chen, et al., Methyl deficient diet aggravates experimental colitis in rats, J. Cell Mol. Med. 15 (11) (2011) 2486–2497.
[40] M.S. Ellulu, et al., Obesity and inflammation: the linking mechanism and the complications, Arch. Med. Sci. : AMS 13 (4) (2017) 851–863.
[41] E. Oken, et al., Gestational weight gain and child adiposity at age 3 years, Am. J. Obstet. Gynecol. 196 (4) (2007) 322 e1-8.
[42] D.A. Lawlor, et al., Epidemiologic evidence for the fetal overnutrition hypothesis: findings from the mater-university study of pregnancy and its outcomes, Am. J. Epidemiol. 165 (4) (2007) 418–424.
[43] R. Xie, et al., Maternal high fat diet alters gut microbiota of offspring and exacerbates DSS-induced colitis in adulthood, Front. Immunol. 9 (2018) 2608–2608.
[44] M.A. Hahn, et al., Methylation of polycomb target genes in intestinal cancer is mediated by inflammation, Cancer Res. 68 (24) (2008) 10280–10289.
[45] H. Wehbe, et al., Interleukin-6 contributes to growth in cholangiocarcinoma cells by aberrant promoter methylation and gene expression, Cancer Res. 66 (21) (2006) 10517–10524.
[46] J.P. Issa, et al., Accelerated age-related CpG island methylation in ulcerative colitis, Cancer Res. 61 (9) (2001) 3573–3577.
[47] T. Maekita, et al., High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosae and its possible association with gastric cancer risk, Clin. Cancer Res. 12 (3 Pt 1) (2006) 989–995.
[48] F.Y. Wang, et al., Aberrant DNA methylation in ulcerative colitis without neoplasia, Hepato-Gastroenterology 55 (81) (2008) 62–65.
[49] K. Tominaga, et al., Prediction of colorectal neoplasia by quantitative methylation analysis of estrogen receptor gene in nonneoplastic epithelium from patients with ulcerative colitis, Clin. Cancer Res. 11 (24 Pt 1) (2005) 8880–8885.
[50] T. Moriyama, et al., Hypermethylation of p14 (ARF) may be predictive of colitic

cancer in patients with ulcerative colitis, Dis. Colon Rectum 50 (9) (2007) 1384–1392.
[51] S. Cui, et al., Folic acid modulates VPO1 DNA methylation levels and alleviates oxidative stress-induced apoptosis in vivo and in vitro, Redox Biol. 19 (2018) 81–91.
[52] A.F. Kolb, L. Petrie, Folate deficiency enhances the inflammatory response of macrophages, Mol. Immunol. 54 (2) (2013) 164–172.
[53] S. Kawanishi, et al., Crosstalk between DNA Damage and Inflammation in the multiple Steps of carcinogenesis, Int. J. Mol. Sci. 18 (8) (2017) 1808.
[54] D.D. Anderson, C.F. Woeller, P.J. Stover, Small ubiquitin-like modifier-1 (SUMO-1) modification of thymidylate synthase and dihydrofolate reductase, Clin. Chem. Lab. Med. 45 (12) (2007) 1760–1763.
[55] D.D. Anderson, et al., Serine hydroxymethyltransferase anchors de Novo thymidylate synthesis pathway to nuclear lamina for DNA synthesis, J. Biol. Chem. 287 (10) (2012) 7051–7062.
[56] X. Guo, et al., Protective effect of folic acid on oxidative DNA damage: a randomized, double-blind, and placebo controlled clinical trial, Medicine (45) (2015) 94 e1872-e1872.
[57] G.P. Basten, et al., Sensitivity of markers of DNA stability and DNA repair activity to folate supplementation in healthy volunteers, Br. J. Canc. 94 (12) (2006) 1942–1947.
[58] S.J. Duthie, et al., Folate deficiency in vitro induces uracil misincorporation and DNA hypomethylation and inhibits DNA excision repair in immortalized normal human colon epithelial cells, Nutr. Cancer 37 (2) (2000) 245–251.
[59] S.J. James, L. Yin, Diet-induced DNA damage and altered nucleotide metabolism in lymphocytes from methyl-donor-deficient rats, Carcinogenesis 10 (7) (1989) 1209–1214.
[60] S.J. Duthie, A. Hawdon, DNA instability (strand breakage, uracil misincorporation, and defective repair) is increased by folic acid depletion in human lymphocytes in vitro, FASEB J. 12 (14) (1998) 1491–1497.
[61] W.Y. Zhu, P.W. Melera, Basal levels of metallothionein I and II expression in mouse embryo fibroblasts enhance growth in low folate through a cell cycle mediated pathway, Cell Biol. Int. 25 (12) (2001) 1261–1269.
[62] M.J. Koury, J.O. Price, G.G. Hicks, Apoptosis in megaloblastic anemia occurs during DNA synthesis by a p53-independent, nucleoside-reversible mechanism, Blood 96 (9) (2000) 3249–3255.
[63] R.-F.S. Huang, et al., Folate deficiency induces a cell cycle-specific apoptosis in HepG2 cells, J. Nutr. 129 (1) (1999) 25–31.
[64] C. Courtemanche, et al., Folate deficiency inhibits the proliferation of primary human CD8+ T lymphocytes in vitro, J. Immunol. 173 (5) (2004) 3186–3192.
[65] A. Skapenko, et al., The role of the T cell in autoimmune inflammation, Arthritis Res. Ther. (2005) S4–S14 7 Suppl 2(Suppl 2).
[66] T. Yamaguchi, et al., Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor, Immunity 27 (1) (2007) 145–159.
[67] M. Kinoshita, et al., Dietary folic acid promotes survival of Foxp3+Regulatory T cells in the colon, J. Immunol. 189 (6) (2012) 2869–2878.
[68] B. Singh, et al., Control of intestinal inflammation by regulatory T cells, Immunol. Rev. 182 (1) (2001) 190–200.
[69] A.C. Peeters, et al., The effect of homocysteine reduction by B-vitamin supplementation on markers of endothelial dysfunction, Thromb. Haemost. 92 (5) (2004) 1086–1091.
[70] P. Dusitanond, et al., Homocysteine-lowering treatment with folic acid, cobalamin, and pyridoxine does not reduce blood Markers of inflammation, endothelial dysfunction, or Hypercoagulability in patients with previous transient ischemic Attack or stroke, Stroke 36 (1) (2005) 144–146.
[71] E.G. Vermeulen, et al., Homocysteine-lowering treatment with folic acid plus vitamin B6 lowers urinary albumin excretion but not plasma markers of endothelial function or C-reactive protein: further analysis of secondary end-points of a randomized clinical trial, Eur. J. Clin. Investig. 33 (3) (2003) 209–215.
[72] Ø. Bleie, et al., Homocysteine-lowering therapy does not affect inflammatory markers of atherosclerosis in patients with stable coronary artery disease, J. Intern. Med. 262 (2) (2007) 244–253.
[73] M. USUI, et al., Endothelial dysfunction by acute hyperhomocyst(e)inaemia: restoration by folic acid, Clin. Sci. 96 (3) (1999) 235–239.
[74] Bellamy, et al., Oral folate enhances endothelial function in hyperhomocysteinaemic subjects, Eur. J. Clin. Investig. 29 (8) (1999) 659–662.
[75] K.S. Woo, et al., Folic acid improves arterial endothelial function in adults with hyperhomocystinemia, J. Am. Coll. Cardiol. 34 (7) (1999) 2002–2006.
[76] T.D. Schaible, et al., Maternal methyl-donor supplementation induces prolonged murine offspring colitis susceptibility in association with mucosal epigenetic and microbiomic changes, Hum. Mol. Genet. 20 (9) (2011) 1687–1696.
[77] S.A. Mir, et al., Prenatal methyl-donor supplementation augments colitis in young adult mice, PLoS One (8) (2013) 8 p. e73162-e73162.
[78] E. Pannia, et al., A high multivitamin diet fed to Wistar rat dams during pregnancy increases maternal weight gain later in life and alters homeostatic, hedonic and peripheral regulatory systems of energy balance, Behav. Brain Res. 278 (2015) 1–11.
[79] R.J. O'Neill, P.B. Vrana, C.S. Rosenfeld, Maternal methyl supplemented diets and effects on offspring health, Front. Genet. (5) (2014) 289.
[80] J. Sauer, J.B. Mason, S.-W. Choi, Too much folate: a risk factor for cancer and cardiovascular disease? Curr. Opin. Clin. Nutr. Metab. Care 12 (1) (2009) 30–36.
[81] S.L. Whittle, R.A. Hughes, Folate supplementation and methotrexate treatment in rheumatoid arthritis: a review, Rheumatology 43 (3) (2004) 267–271.
[82] M.T. Nurmohamed, Cardiovascular risk in rheumatoid arthritis, Autoimmun. Rev. 8 (8) (2009) 663–667.
[83] R. Obeid, The metabolic burden of methyl donor deficiency with focus on the betaine homocysteine methyltransferase pathway, Nutrients 5 (9) (2013) 3481–3495.
[84] P. Frosst, et al., A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase, Nat. Genet. 10 (1) (1995) 111–113.
[85] M. Hiraoka, Y. Kagawa, Genetic polymorphisms and folate status, Congenital. Anom. 57 (5) (2017) 142–149.
[86] L. Bathum, et al., Genetic and environmental influences on plasma homocysteine: results from a Danish twin study, Clin. Chem. 53 (5) (2007) 971–979.
[87] H. Refsum, et al., The hordaland homocysteine study: a community-based study of homocysteine, its determinants, and associations with disease, J. Nutr. 136 (6) (2006) 1731S–1740S.
[88] L. Peyrin-Biroulet, et al., Association of MTRR 66A > G polymorphism with superoxide dismutase and disease activity in patients with Crohn's disease, Am. J. Gastroenterol. 103 (2) (2008) 399–406.
[89] X. Qin, et al., MTHFR C677T and MTR A2756G polymorphisms and the homocysteine lowering efficacy of different doses of folic acid in hypertensive Chinese adults, Nutr. J. 11 (2012) 2.
[90] C. Antoniades, et al., MTHFR 677 C > T Polymorphism reveals functional importance for 5-methyltetrahydrofolate, not homocysteine, in regulation of vascular redox state and endothelial function in human atherosclerosis, Circulation 119 (18) (2009) 2507–2515.
[91] K.P. Shelnutt, et al., Methylenetetrahydrofolate reductase 677C– > T polymorphism affects DNA methylation in response to controlled folate intake in young women, J. Nutr. Biochem. 15 (9) (2004) 554–560.
[92] S. Friso, et al., A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status, Proc. Natl. Acad. Sci. U. S. A. 99 (8) (2002) 5606–5611.
[93] N. Botto, et al., Genetic polymorphisms in folate and homocysteine metabolism as risk factors for DNA damage, Eur. J. Hum. Genet. 11 (9) (2003) 671–678.
[94] M. Kimura, et al., Methylenetetrahydrofolate reductase C677T polymorphism, folic acid and riboflavin are important determinants of genome stability in cultured human lymphocytes, J. Nutr. 134 (1) (2004) 48–56.