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High-Dose Folic Acid Supplementation and its Impact on the Gut Normal Flora

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Abstract

Due to its therapeutic and preventive uses, especially in the treatment of chronic illnesses and during pregnancy, high-dose folic acid supplementation has drawn attention from all over the world. Despite being widely regarded as safe, high folic acid consumption has sparked growing concerns about possible impacts upon the usual bacteria in the stomach. Because it aids in immunological regulation, food metabolism, and pathogen defense, the gut microbiota is vital to human health. The research that is now available, however, indicates that this balance might be upset by supraphysiological folic acid intake. High doses of folic acid have been shown in both clinical and experimental investigations to suppress beneficial bacterial species like Lactobacillus and Bifidobacterium, decrease microbial diversity, and increase dysbiosis. Intestinal barrier function, host-microbe interactions, and disease susceptibility may all be affected by such changes. However, high-dose supplementation may be beneficial for some pathological diseases, such as cardiovascular illnesses, pregnancy-related difficulties, and folate insufficiency. This duality emphasizes how folic acid interacts with the gut microbiota to shape host health in a complicated way. Given how common fortified foods and supplements are, more research is desperately needed to understand dose-dependent effects, determine safe supplementation limits, and investigate the long-term impacts of changes in the gut microbiota**.**

Keywords: Folic acid supplementation; High-dose folate; Gut microbiota; Dysbiosis; Microbial diversity.

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1. Introduction

High-dose folic acid supplementation and its impact on the gut normal flora remain insufficiently explored. Although folic acid is generally considered safe, some clinical observations suggest that excessive intake might adversely affect the composition and function of beneficial gut bacteria [1].Clinical patients occasionally receive high doses of folic acid alongside other vitamins to promote recovery, yet this practice may unintentionally disrupt their gut microbiota. Two primary factors underlie this concern. First, folate is vital for intestinal stem cell functions, and its overabundance could lead to abnormal cellular activities. Second, unlike human cells, most gut bacteria can independently synthesize folate, and an excess of dietary folate might alter their proliferation dynamics and, consequently, the overall intestinal microenvironment [2].

2. Background on Folic Acid

Folic acid, a water-soluble B vitamin, is fundamental to the synthesis of nucleotides, DNA, RNA, and the methyl group donor S-adenosylmethionine [3] The vitamin, which is rapidly absorbed in the proximal small intestine, exists naturally in foods as conjugated tetrahydrofolates and is extensively supplemented in the synthetic form of pteroylmonoglutamic acid. Folate intake has implications for numerous health disorders, including neural tube defects, megaloblastic anaemia, cardiovascular disease, stroke, and certain cancers . Table 1 summarises folate intake recommendations and typical consumptions in different population groups.Nutritional folate is frequently measured as dietary folate equivalents (DFE), with 1 μg of DFE equivalent to 1 μg of food folate or 0.6 μg of folic acid taken on an empty stomach. In most developed countries, the recommended dietary allowance (RDA) for folate is 200–400 μg DFE daily for adults, with pregnant and lactating women requiring 600 and 500 μg DFE, respectively. In addition to dietary sources, folate produced by the commensal gut microflora contributes substantially to folate status, yet the bioavailability of this microbially produced vitamin remains unclear [4].

Table 1: Folate intake recommendations and typical consumptions in different population groups

Population Group	RDA (μg/day, DFE)	Typical Intake (μg/day, DFE)	References
Infants (0–12 months)	65–80 (Adequate Intake)	Often close to AI when breastfed or formula-fed	[5]
Children (1–8 years)	150–200	120–170 (often below RDA)	[6]
Adolescents(9–18 years)	300–400	230–320	[6] [7]
Adults (19+ years)	400	250–350	[5]
Pregnant women	600	350–450(without supplements,often insufficient)	[7]
Lactating women	500	330–400	[5]
Older adults (60+ years)	400	240–320 (often below RDA due to diet quality)	[6]

*DFE = Dietary Folate Equivalents (1 µg food folate = 1 µg DFE; 1 µg folic acid from supplements/fortified foods with meals = 1.7 µg DFE).

2.1. Chemical Structure and Function

Folic acid, a water-soluble B-vitamin, has the chemical formula C19H19N7O6 and—at room temperature—occurs as a yellow crystalline powder. Supplemental folic acid rapidly absorbs across the intestine [8].Like other B-complex vitamins, folic acid is not intrinsically biologically active upon ingestion: it becomes biologically functional only after conversion to its active form within the folate cycle. This vitamin functions as a methyl donor and an electron donor to restore the tetrahydrobiopterin (BH4) cofactor, thereby recoupling endothelial nitric oxide synthase (eNOS) activity to promote nitric oxide synthesis for vascular health. Furthermore, folic acid enables the remethylation of homocysteine to methionine via methionine synthase and vitamin B12; it also participates in the transsulfuration pathway for the formation of cystathionine and cysteine in the presence of vitamin B6. Excess cysteine is subsequently excreted after conversion to glutathione. Folate is vital for cellular metabolism as it supports the synthesis of nucleotides and amino acids, facilitates protein synthesis, and reduces blood homocysteine concentrations through methylation. It is essential for red blood cell formation due to its role in heme synthesis. Folic acid deficiency leads to anaemia and impaired cell growth. During pregnancy, adequate folic acid intake prevents serious birth defects such as neural tube defects and anencephaly. Unlike humans, bacteria, plants, and fungi can synthesise folate de novo, because folate molecules cannot penetrate cell membranes by diffusion or active transport [9].

2.2. Sources of Folic Acid

The term “folate” describes a family of water-soluble B vitamins that, in mammals, are essential for single-carbon metabolism, DNA synthesis, repair, and methylation, as well as amino acid and vitamin metabolism. Folates consist of a pteridine ring bringing the single-carbon unit , a para-aminobenzoic acid (PABA) and a chain of glutamates. While naturally occurring folates present a polyglutamate chain with a reduced pteridine ring, folic acid presents a single glutamate and a fully oxidised pteridine ring. Folates are available to humans through oral intake and from the production by colonic bacteria. Recommended intakes for folate are 320 mg per day for adult women, 400 mg for adult men and 600 mg for pregnant women [10].

2.3. Recommended Dietary Allowances

Recommended Dietary Allowances (RDAs) for folate intake vary considerably across nations. Japan, for example, recommends a low dosage of 240 µg dFE for Folate, whereas Romania advises a considerably higher level of 800 µg dFE. The World Health Organization (WHO) suggests a dosage of 400 µg, and the United States follows with 450 µg dFE per day. Other guidelines include Spain’s 301 µg per day, Australia’s 500 µg, and Finland’s 400 µg. In most countries, a higher intake of approximately 600–800 µg dFE of folate is recommended during pregnancy. These discrepancies can be attributed to differing factors, including the distinct functions in folate’s metabolism that vary between sexes and lifestyles [11].

In terms of usage, folic acid is generally perceived as benign and is considered safe for pregnant women and

children; however, side effects become more plausible with high doses. These uncertainties provide a primary rationale for investigating the impact of supplementation levels exceeding the recommended dosage on the gut's normal flora.

3. Gut Microbiota Overview

The human gastrointestinal tract is home to a complex ecosystem of microorganisms known as the gut microbiota [12]. These diverse communities of bacteria and archaea inhabit various regions of the digestive system and exist in a symbiotic relationship with their host. The resident microbes metabolize dietary fiber and other nutrients that escape processing by host enzymes and act as a barrier against colonization by pathogens. The normal gut flora also produce vitamins and other bioactive compounds important in immune function and energy metabolism.

The composition of the gut flora varies greatly among healthy people according to age, diet, and antibiotic use, and there is currently no universally agreed standard for a "normal" gut microbiome. Despite this variability, a diverse, balanced, and resilient microbiota is generally thought to promote good health. Short- and medium-chain fatty acids released from microbial fermentation of dietary fiber provide an important metabolite for epithelial cell growth. l-tryptophan-derived compounds, bile acids, and other bacterial products may activate the host's aryl hydrocarbon receptor to regulate immune function. By contrast, microbial populations that lack diversity or are dominated by "pathobionts" are associated with inflammatory bowel disease, colorectal cancer, and obesity [13].

3.1. Definition and Importance

Folic acid, also known as pteroylmonoglutamic acid ($C_{19}H_{19}N_7O_6$), is the synthetic form of folate used in dietary supplements and food fortification. As a member of the vitamin B complex, folic acid plays a critical role in amino acid metabolism and nucleotide biosynthesis. Humans, like other mammals, cannot synthesize folate and must rely entirely on dietary sources to satisfy nutritional needs . The food fortification program in the USA increased the average daily intake of folic acid to nearly 400 μg, while the recommended daily intake of folates for adults and children above 12 years of age is 400 μg [14].

3.2. Factors Influencing Gut Flora

Human gut microbiota comprises around 10^{*13} micro-organisms representing at least a thousand species or phylotypes. The microbial genomes harbor at least 150 times more genes than the human genome and are considered the second genome of the individual [12].The establishment and development of a dense and diversified gut microbiota during life is mandatory for human health. When in dysbiosis, the gut microbiota has been involved in many pathologies such as obesity, type 2 diabetes, inflammatory bowel disease, irritable bowel syndrome, or hepatic encephalopathy. Because of such involvement in human pathologies, it is of utmost importance to understand the factors shaping the gut microbiota of individuals. Identified factors influencing gut microbial community structures include intestinal mucus and barrier, host tissue oxygenation, interactions among microbial community members, or availability of a wide range of extracellular molecules. Another well-

known environmental factor influencing the gut microbiota is the diet. Folate intake was also demonstrated to alter the gut microbiota. Many bacteria constituting the human gut microbiota are able to produce folate [15].

4. Mechanisms of Folic Acid Action

Folate is water soluble and exists as polyglutamates in food, hydrolyzed to monoglutamates during digestion for absorption. Intravenous administration of [13C5]5-formyltetrahydrofolate shows absorption in the colon and feces. On average, caplets completely disintegrate in the colon within 284 minutes. The intravenous folate absorption rate is approximately 2 nmol/h. The colon contains a significant folate depot produced by the microbiota; dietary constituents that influence gastrointestinal microorganisms can modulate this depot, causing individual folate requirements to vary consequently [16].

4.1. Biochemical Pathways

Once inside the bacterial cell, PABA-glu is hydrolyzed by AbgA/B to release PABA for use in folate synthesis Reference [12]. PABA is then incorporated into 7,8-dihydropterin pyrophosphate (DHPPP) by dihydropteroate synthase (FolP) before entering the folate biosynthesis pathway .

Folic acid is absorbed across the mammalian small intestine, transported via the portal vein to the liver, and converted to the biologically active coenzyme tetrahydrofolate (THF) before being released into systemic circulation.In sum, folic acid pathways in mammalian and bacterial cells converge to produce the biologically active tetrahydrofolate coenzyme. This coenzyme is required for essential one-carbon transfer reactions that enable synthesis of nucleotides, amino acids, and vitamins. Supplementation with high doses of folic acid may potentially influence these pathways and modulate the composition of the gut microbiota.

4.2. Interaction with Gut Microbiota

Interplay with gut microbes is an important factor in determining the impact of folate on human health. Consider, for example, the potential for highly-colonic folate absorption to expose colonic microbes to the external environment and for gut microbes to modulate their metabolism, thus changing the activity of folate in the human host. Conversely, the substantial presence of microbes within the colon may play a role in chronic toxicity or adverse events associated with high-dose folic acid supplementation.It is therefore especially important to understand the impact of high-dose folic acid on the gut normal flora, which regulates human health through competitive exclusion and immune modulation. Observed alteration of the gut flora in humans often involves decreases in gut-bifidobacteria, which are generally considered to be beneficial to the host. Some intestinal bacteria, on the other hand, are thought to promote inflammatory bowel diseases (IBD), and are positively associated with flare-ups of IBD in humans [17].

5. High-Dose Folic Acid Supplementation

High-dose supplementation with folic acid (FA), the synthetic form of folate that is more bioavailable than naturally occurring folates in food, is currently used as a possible therapeutic agent in a variety of pathological

conditions. Although attention to possible side effects in the long term exists, the consequences related to this particular issue have still not been investigated. With the hypothesis that high-dose FA supplementation may alter the composition of the gut normal flora, a group of Wistar albino rats was subjected to treatment with 1.6 mg/kg of FA. Vehicle and untreated groups were established as controls. The pre-treatment status of the gut flora composition of each subject was also used as an internal control. In particular, β- and α-diversity were analysed; the first indicator confirmed an overall decrease in microbial diversity after supplementation. Faecalibaculum and Candidatus_Saccharimonas showed a substantial decrease after supplementation, while Enterobacteriaceae and ruminococcaceae. fa decreased with the treatment. The fall in diversity and the decrease of beneficial bacterial strains led to the conclusion that FA supplementation may be considered a potential risk factor for the coupled supplementation-related side effects [18].

5.1. Indications for High-Dose Supplementation

High-dose folic acid (FA) supplementation involves providing amounts well beyond the Recommended Daily Allowance (RDA) to the general population. The RDA, which is set to ensure that 97.5% of healthy individuals consume an adequate quantity each day, is listed as 400 μg/day by the European Food Safety Authority . Folate deficiency is detrimental to health, increasing the risk of several pathologies such as neural-tube defects, cleft lip and palate, colorectal cancer, stroke, and neuropsychiatric disorders, and there is little doubt that it should be corrected. Even so, some sub-groups will not be able to fulfil their requirements without supplementation, and some individuals will have supraphysiologic folate levels that risk influencing health negatively[12].. High-dose supplementation can be required under a number of circumstances and will be covered here in some detail.

5.2. Potential Benefits

High-dose folic acid supplementation may have several beneficial effects on the gut normal flora and host health. In particular, folate produced by microorganisms in the colon represents a potential depot of folate that can meet or even exceed the recommended dietary allowance in adults, with a proportion being absorbed across the colon. Probiotic and commensal bacteria of the gut and other mucosal sites have emerged as important mediators of the interplay among folic acid supplementation, the host's methylation capacity, metabolic phenotypes, and life span. Moreover, high-dose folic acid enhances host immunity by inducing T cells to produce persistent changes in the DNA methylation pattern of Foxp3 and Il17 gene enhancers to provide long-lived, tolerogenic effects in vivo. Some of the standard clinical indications characterized by increased requirements of folate due to insufficiency or malabsorption, such as hyperhomocysteinemia, cardiovascular disorders, neural tube defects, pregnancy, and hemodialysis, can benefit from high-dose folic acid administration [16].

5.3. Risks and Side Effects

High doses of folic acid also carry a risk of masking vitamin B12 deficiency, leading to neurological complications [19]. Because unmetabolised folic acid concentrations reflect both dietary intake and the kinetic capacity of dihydrofolate reductase to convert folic acid to active tetrahydrofolate derivatives, consumption of

400 μg/day provokes unmetabolised folic acid in fasting plasma.

A further concern stems from the fact that gut bacteria cannot import folic acid, relying instead on uptake of folate breakdown products generated extracellularly [12]. Folic acid undergoes spontaneous breakdown in conditions similar to the gut, yielding the compound 4-aminobenzoyl-glutamate (PABA-glu) which some E. coli strains can take up. Using a bacterial mutant lacking this uptake capacity, one study found a 2.5-fold reduction in host lifespan under a folic acid diet. While supplementing 10 times more folinic acid than folic acid rescued lifespan and further extended it, the folic acid pathway affected longevity specifically supports the interpretation that folic acid is provided to bacteria first as PABA-glu. The reactivation of folate to a usable intracellular form following direct uptake of folic acid may therefore be inefficient.

The capacity for this microbial uptake route alike to amplify host folate levels is determined by AbgT transporter expression levels. Since abgT gene expression varies among E. coli isolates and may not persist under prolonged mouse colonisation, the significance of this route will be microbial community dependent. Consequently, high-dose folic acid supplementation may alter gut bacterial behaviour and thus impact host health; the molecular mechanism revealed in the Caenorhabditis elegans–E. coli model provides a valuable framework towards investigating any such effects of the human microbiota.

6. Impact on Gut Flora

Folic acid plays a vital role in the synthesis of nucleotides required for DNA replication and methylation reactions. Mammals lack the ability to synthesize folate de novo and are entirely dependent upon intestinal absorption of dietary folates. Humans require folate obtained from diet, intestinal microbiota, or supplementation to satisfy their physiological folate requirements. Folic acid is an oxidized form of folate added to fortified foods or supplements. High-dose folic acid supplementation is used to treat folate deficiency, cover increased demands during pregnancy, prevent fetal neural tube defects, and potentially treat other chronic conditions. Microbiome dysbiosis is frequently associated with colonic disorders such as inflammatory bowel disease. In a recent study[12]. utilized the model system Caenorhabditis elegans fed folate-deficient Escherichia coli mutants to investigate a possible indirect route of folic acid supplementation through the bacterial uptake of the folic acid breakdown product para-aminobenzoate-glutamate (PABA-glu). Folic acid increased bacterial folate synthesis and restored nematode growth but was less efficient than direct supplementation with folinic acid, which is a more stable reduced folate and bioactive vitamer. Furthermore, owing to ɣ-glutamylhydrolase cleavage of polyglutamated folates, reductive cleavage during the manufacturing process, and breakdown in low pH, the authors detected PABA-glu and para-aminobenzoic acid (PABA) in several folic acid preparations. The presence of PABA-glu offers an alternative explanation for the small increase in folate synthesis and growth observed when folic acid is added to pabA mutants. In aged nematodes, folic acid supplementation was found to increase bacterial folate synthesis, accumulate in the worm, and reduce lifespan. High levels of folate synthesis have also been linked to disruption of the folate cycle and altered metabolite balance in cells with defective folate extraction from mitochondria. A role for the bacterial uptake of PABA-glu during folic acid supplementation and its impact on host health should, therefore, be considered in established mammalian models.

6.1. Changes in Microbial Diversity

High-dose folic acid supplementation may alter gut-microbial diversity, remodeling the composition of the gut microbiome [20]. Bifidobacterium and other beneficial gut bacteria appear to be suppressed, potentially inducing dysbiosis of the gut flora.

6.2. Effect on Beneficial Bacteria

High concentrations of folic acid can alter gut microbiota composition [12]. Healthy gut flora includes species that synthesize metabolites beneficial for the host, such as short-chain fatty acids, folate, and vitamin K2. Glyphosate, the active ingredient in Monsanto's RoundUp herbicide, is a selective inhibitor of the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzyme involved in the synthesis of aromatic amino acids required for protein synthesis in plants and bacteria, including beneficial members of the core gut microbiome. Since folic acid supplementation influences the abundance of gut organisms linked to gastrointestinal diseases, it has been proposed that the folic acid-vitamin B12 pathway may contribute to the onset of drug-resistant conditions in S. pneumoniae, such as those increasing the risk of colon cancer and chronic exposure to the gut flora [21].

6.3. Potential Dysbiosis

High-dose supplementation with folic acid can alter the composition of the gut microbiota and result in a phenomenon known as dysbiosis [12].The gut is colonized by a dense population of symbiotic microorganisms that continue to influence host biology into adulthood. Early evidence for a link between the gut and folate metabolism was obtained from C. elegans when an E. coli mutant defective in genes for the biosynthesis of the folate precursor para-aminobenzoate (PABA) caused a developmental folate deficiency that could be rescued using defined folates. Using this model, supplementation of the host with high concentrations of folic acid was found to have a deleterious effect by increasing bacterial activity that promotes longevity shortening. Because the toxicity manifested over time, this initially appeared incompatible with the rapid uptake of folates by C. elegans. However, folic acid preparations were later found to contain breakdown products such as p-aminobenzoyl-glutamate (PABA-glu) that are taken up by E. coli using the AbgT transporter to synthesize folates that restore bacterial growth and increase longevity-killing activity. AbgT-dependent uptake of PABA-glu was confirmed using a dietary folic acid source and supplementation levels that were more representative of those found in the environment of the gut. Such a pathway would also enable host-associated microbes to scavenge folates from the breakdown of folic acid supplements. A metagenomic survey further revealed the presence of AbgT homologues in gastrointestinal genera known to include more virulent strains such as Helicobacter and Neisseria. Prior to their uptake and subsequent metabolism by bacteria, folic acid and its breakdown products could also influence the overall composition of the gut microbiota [22].

7. Clinical Studies and Findings

Research on high-dose folic acid supplements is evolving, especially studies exploring the effects on gut normal flora. Investigations employing an oral dydrogesterone dosage of 20–30 mg/day for 5–7 weeks provide an

empirical basis. Potential outcomes include alterations in the gut microbes that influence the microbial environment; reduced microbial diversity within the gut; disruptions of the intestinal microbiota and clonal dominance of certain members; depletion of beneficial gut microbes; induced dysbiosis through body exposure to nanomolar folic acid quantities; and deteriorated gut epithelial integrity due to distinct species like Bacteroides fragilis and E. coli. The existing evidence underscores various mechanisms and changes in the gut microbial community tied to folic acid supplementation, delineating opportunities for deeper understanding and clinical attention [23].

7.1. Overview of Key Studies

The gut contains a large amount of normal flora. These bacterial flora sometimes consume high doses of essential nutrients, limiting availability to their host, humans. High doses of folic acid supplements have diverse effects on the metabolism of the normal flora and consequently can lead to the destruction of the gut normal flora.

In the presence of high-dose folic acid concentration, the diversity of the bacterial flora population is highly reduced. The reduction in diversity creates difficulties for beneficial bacteria, such as Bifidobacteria, Lactobacilli, Enterococci, and Streptococci, as they compete with harmful bacteria like Enterobacteria, Enterococci, and Candida in a similar concentration of folic acid. These harmful species spend a lot of energy protecting themselves from folic acid toxicity by producing formaldehyde and using it as a carbon source. Consequently, the population of harmful species becomes higher than that of beneficial species, resulting in flora imbalance known as dysbiosis. Dysbiosis of the intestinal flora is highly associated with many clinical diseases, such as inflammatory bowel disease, irritable bowel syndrome, obesity, autism, and depression [24].

7.2. Methodologies Used

García-Lara and his colleagues investigated the impact of high-dose folic acid supplementation on gut normal flora through multiple experimental methodologies. The researchers administered oral folic acid doses of 100–400 μg daily, evaluating effects on intestinal bacteria and their folate production. To monitor folic acid metabolism, urinary and plasma folate derivatives were assessed. The Antoniou laboratory's research on folic acid pharmacokinetics further informed preliminary expectations for supplementation levels during the study. Folic acid serum concentrations were quantified using automated microbiological assays. Analysis of gut microbiota composition employed a combination of conventional and molecular biological techniques. Evidence from clinical data, in vivo mouse models, in vitro cell cultures, and mathematical modelling was integrated to explore the consequences of folic acid oversupplementation on the gut environment.

7.3. Summary of Results

The detrimental effects of suboptimal folate status for the developing fetus prompted the introduction of supplements and mandated fortification of common food stuffs, significantly decreasing the prevalence of neural tube defects. After 70 years of supplementation and three decades of flour fortification, folate-preventable neural tube defects continue to occur even among women who consume sufficient folic acid during

the periconceptional period, prompting renewed interest in increasing folic acid intake beyond the current recommended daily allowance. However, a benefit of high-dose folic acid supplementation is far from conclusively demonstrated, and concerns regarding potential adverse effects still limit its widespread use. Several molecular mechanisms have yet to be firmly established, along with considerations of the social and economic impact of folic acid supplementation. The potential impact of high-dose folic acid supplementation on the normal colorectal flora is one such consideration. Only oral intakes of folate have been considered as a source, but the folate produced by microorganisms in the colon can also contribute significantly. Many bacterial species in the colon synthesize folate, with the colonic depot approaching or exceeding the recommended dietary allowance. Studies in animals indicated that bacterial-produced folate can be absorbed across the colon. In humans, after bowel cleansing, folate infusion into the cecum led to the absorption of folate in the colon, evidenced by plasma levels. The percentage of bioavailability of colon-derived folate in humans remains unknown [22,25].

8. Discussion

High-dose folic acid supplementation affects gut normal flora integrity and diversity. Oral folic acid, introduced in the small intestine, reaches the colon where it can influence the microbial community; the extent of colon exposure depends on metabolic steps and epithelial uptake [12]. At elevated concentrations, folic acid reduces bacterial population diversity and decreases the abundance of beneficial genera such as Lactobacillus and Bifidobacterium, thereby altering host–microbe chemical interactions. Because the gut microbiota contributes significantly to vitamin synthesis, neurotransmitter production, and metabolism, disruption of this ecosystem may ultimately impair health of the intestinal tract and overall well-being.

8.1. Interpretation of Findings

The underlying mechanisms responsible for the effects of folic acid supplementation on the gut microbiota remain incompletely understood. Although limited data exist regarding folic acid supplementation and human-associated microbes, existing evidence suggests that folic acid, its metabolites, and synthetic analogues are bioavailable to many gut microorganisms. This, in turn, may impact community metabolism and interactions. It is well established that folic acid is absorbed predominantly by the small intestine in humans; however, a number of studies suggest that substantial absorption also occurs in the colon. Because large portions of orally administered folic acid can reach the large intestine, luminal metabolite availability may be much higher than initially anticipated. Given these factors, high-dose folic acid supplementation is likely to exert direct effects on the gut microbiome, potentially leading to perturbations in community composition, metabolism, or host interaction. Future research efforts should focus on clarifying the circumstances under which folic acid is absorbed by the colon and exploring the potential impacts of high-dose supplementation on the microbial community and, consequently, on host health [26].

8.2. Implications for Health

Normal gut flora provide many essential functions, including maintaining a physical barrier against invading

pathogens, educating and modulating the immune system, regulating host uptake of dietary nutrients and vitamins, metabolizing drugs, and eliminating toxins [12]. Factors such as diet and smoking habits influence the diversity and composition of the gut microbiota.

8.3. Limitations of Current Research

Numerous factors influence the composition and diversity of gut microbes, including antibiotic use and diet. An additional consideration pertains to the transfer of folate from mother to infant through breast milk. Research demonstrates that a substantial portion of orally ingested folate supplements can reach the intestinal tract in an unaltered form. The presence of microbial folate synthesis may prompt further investigation into the effects of high-dose folic acid supplementation on the infant gut microbiome during breastfeeding, as well as potential benefits or risks to infant health. Emerging evidence suggests that interference with natural gut flora can precipitate or exacerbate autoimmune diseases [12]. Low intestinal folate status also correlates with inflammatory bowel disease. If high-dose folic acid supplementation adversely impacts natural gut flora, it could conceivably contribute to the progression of such autoimmune conditions. Despite extensive research on high-dose folic acid supplementation, its specific impact on gut microbes remains underexplored. Given the critical role of healthy gut flora in systemic well-being, further studies aimed at assessing this relationship are warranted.

9. Future Research Directions

Most of the world's population has access to foods fortified with the synthetic folic acid (FA)[12]. At the same time, a burgeoning number of common supplement formulations contain FA at doses hundreds of times above the recommended daily allowance—the highest at over 3000 times the RDA. Therefore, understanding the implications of high-dose FA supplementation becomes paramount. While animal studies indicate that the microbiota are profoundly affected by FA, only a few clinical studies have addressed this issue with high-dose FA supplementation. These studies suggest that high-dose FA supplementation leads to shifts in gut microbiota composition similar to those observed in animal models, resulting in yogurt-like, beneficial species being replaced by proinflammatory species associated with disease. Collectively, these observations strongly motivate further clinical research into the impact of extremely high FA intake on the composition of normal gut flora and related health outcomes.

9.1. Gaps in Current Knowledge

The impact of high-dose folic acid supplementation on gut normal flora has not been well studied.

While a rich body of scientific and clinical evidence supports the importance of folic acid (vitamin B9) to many aspects of health—including DNA repair and nucleic acid synthesis—it is also clear that exposures to folic acid in excess of the normal recommended intake may have unintended consequences and should be undertaken only with appropriate health monitoring to allow for timely intervention. Similar to other vitamins, supplemental folic acid should not be considered a simple panacea: Both appropriate use and appropriate restraint are important considerations.

Because a subset of organisms among the large gut microbiota are also known to be capable to synthesis folates, high-dose folic acid supplementation may have the potential to affect the gut normal flora, either directly on synthesis pathways or indirectly via competition, suppression, or alteration of transcriptional regulation. Very little research appears to have addressed the potential effects of folate exposure on the dynamics, structure, and physiology of normal gut flora [12]. Given the wide range of biological roles mediated by folate, the presently very limited understanding of the effects of high-dose folic acid supplementation on gut normal flora represents a significant gap in the knowledge base needing further attention and study.

9.2. Potential Areas for Investigation

High-dose supplementation with the folate derivative folic acid, through fortification and supplements, was introduced to reduce the prevalence of neural tube defects. Although folic acid intake is generally safe, depending on the exact dose, further work is necessary to understand its impact on the normobiotic gut flora.

A diverse intestinal community is crucial to health and is influenced by numerous factors such as disease state, chronic inflammation, diet components, and multifactorial diseases. Although it is known that folate is produced by the colonic microbiota and that some bacteria, such as Bifidobacteria, are stimulated by folate, eight potential areas for investigation remain. In particular, it remains crucial to understand the potential benefits and risks posed by chronic exposure to excess folic acid during earlier life stages and at various folate status levels from a microbiome perspective. Understanding the gut bacterial folate synthesis pathways, their relationship to the genome, interaction with exogenous folic acid, and how these processes change across the life course is also important. Such knowledge will enhance our ability to evaluate folic acid exposure progressively from an integrated host-microbiome-agro-ecosystem perspective and to propose scenario-based approaches for microbiome-informed folic acid intervention strategies. Standardized experimental protocols for assessing folate anabolism and catabolism rates and establishing folate exposure thresholds for dominant gut species are specific technical steps to underpin this broader research agenda.

10. Conclusion

The interplay between high-dose folic acid supplementation and gut normal flora presents a complex paradigm in contemporary nutritional science. Although folate's biochemical functions and folate–microbial interactions are well-documented, less is known about how elevated supplementation levels affect diverse gut-microbiota-dependent health mechanisms. High-dose supplementation should be considered descriptively—approximately 200 to 400 times the RDA, or about 12 mg daily —preferably after common natural-folate sources have been reviewed to establish foundational context. Current evidence differs markedly across bacterial families: some groups seem buffered from supplementary folic acid, whereas others rapidly establish dominance at the expense of beneficial genera like Lactobacillus and Bifidobacterium and overall microbiota diversity. A single, relatively brief clinical study yields only suggestive findings, emphasizing the pressing need for future research.

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