

Raising Concerns About Unmetabolized Folic Acid

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Summary

The advent of food fortification with folic acid along with the growing enthusiasm for vitamin supplements have made it quite possible, if not probable, that large populations in several countries are at risk for high intakes of synthetic folic acid. The ramifications of this are unknown, but there is a growing body of evidence which suggests that there may be the risk of serious health problems associated with this practice. Of particular concern are the differences in metabolism between dietary folates and synthetic folic acid and the potential of the latter to yield high levels of unmetabolized folic acid in the circulation. This phenomenon appears to be largely unrecognized, and the potential risks largely ignored. The concerns that have been identified relate mostly to cancer and cognition.

Introduction

Human intake of folate/folic acid is essentially limited to four sources: food, supplements including multivitamins, therapeutic doses taken on the advice of physicians, and fortified foods. It is becoming more apparent that naturally occurring folate in foods must be clearly differentiated from folic acid which is used in supplements and in food fortification, the reason being that they are not in general equivalent. The term folic acid will be used to represent pteroylmonoglutamate (PGA), the synthetic chemical used in fortification, therapy and supplements, and folate to represent natural forms from food sources, both primary as downstream metabolites. While it is true that folic acid (PGA) is converted to

metabolites identical to those obtained from food sources, what is significant is that this process saturates at folic acid intake levels between 200 and 400 $\mu\text{g}/\text{day}$ and at intakes above this saturation limit, unmetabolized folic acid (UMFA) appears in the circulation.^{1,2} This does not seem to be common knowledge. Folic acid and dietary folates are frequently viewed as the same substance. It has also only recently been observed that while dietary folate is initially metabolized in the small intestine, the liver is the primary site for the metabolism of folic acid.³ The overall and long-term implications are unknown.

There is a potential problem for the following reasons: (a) no long-term information is available on the safety or possible adverse effects of UMFA or high intakes of folic acid in general; (b) the growing and widespread use of multivitamin supplements as well as B-vitamin supplements increases the likelihood of significant as well as very high levels of UMFA; (c) in countries such as the USA, Canada and a few others, mandatory and voluntary food fortification offers the opportunity for significant intake of folic acid from prepared foods such as bread and ready-to-eat breakfast cereals; (d) there is a growing body of evidence suggesting that high levels of folate/folic acid intake may increase the risk of various disorders including cancer. High intakes of folate/folic acid can only be achieved with multivitamins, separate folic acid supplements, or high consumption of folic acid fortified foods. Thus high folate/folic acid intake may be much more common than generally recognized and will invariably be associated with circulating UMFA. This commentary will address some of these issues.

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The Range of Folate and Folic Acid Intakes

It is well known that for individuals who do not supplement and do not eat fortified foods, dietary intakes of folate are in the 200-400 $\mu\text{g}/\text{d}$ range. Most North American multivitamins contain 400 μg of folic acid per pill, and many individuals take two per day. Some consider the B vitamins so important they take a so-called B-50 supplement which contains 1 mg folic acid per pill. Sometimes this is in addition to a multivitamin. Food fortification was expected to provide 100-200 $\mu\text{g}/\text{d}$ of folic acid, but there is now evidence that the amount may be considerably higher,⁴ and the opportunity exists for rather high intakes by those who fancy large servings of ready-to-eat breakfast cereals, i.e. intakes of well over 400 μg , the amount per serving, just from this single source. Nutritional drinks and bars can also supply 400 μg per serving. Thus it is possible for intakes from combined sources to reach 1200-1500 $\mu\text{g}/\text{d}$ and in some cases even higher levels can be envisioned, most of which ends up as UMFA. There is little concern, aside from that expressed in a few editorials and perspectives,⁵⁻⁸ about such folic acid intake since the conventional wisdom holds that folic acid is safe; physicians prescribe therapeutic doses of up to 5 mg/d and 1-5 mg/d is common in many intervention studies.

There are a number of incentives that encourage supplementation. Widespread concern among both health care professionals and the general public over the connection between cardiovascular disease risk and homocysteine levels has prompted considerable interest in folic acid supplementation, which will generally produce 20-50% reductions in serum homocysteine, and there have been a number of intervention studies in this context which generally involved doses in excess of 1 mg/d. All women of childbearing age have for some time

been strongly encouraged to take folic acid, typically at a dose level of 400 $\mu\text{g}/\text{d}$, in order to reduce the risk of the folate related neural tube birth defects. This was of course the reason for mandatory food fortification since educational programs designed to motivate women to maintain a satisfactory folate status prior to conception were notoriously ineffective. It is not clear if some women concerned about birth defects in their offspring take a folic acid supplement in addition to a multivitamin, or that much thought is even given to multiple sources of folic acid, either by this group or for that matter by anyone other than a few academics. The USA Institute of Medicine recommendation of an upper tolerable folic acid intake for adults is 1 mg/d from supplements and fortified food and 300-400 $\mu\text{g}/\text{d}$ for children between the ages of 1 and 8. These limits are almost 10 years old and were devised mainly to avoid masking anemia and missing the neuropathy attributed to vitamin B₁₂ deficiency.

Thus it is clear that in some countries there is a strong possibility that high or even very high levels of folic acid intake and circulating UMFA are common. In what follows, evidence will be reviewed relating to why this should be of concern to physicians, those involved in public health, those concerned with both mandatory and voluntary food fortification, and ultimately the general public.

The Cancer Connection

Folate is thought to play a role in cancer prevention. Evidence derived from epidemiologic studies consistently shows positive associations between low levels or intakes of dietary folate and the risk of colon cancer or cancer in other parts of the gastrointestinal tract.⁹ Observational and interventional studies of the relationship between higher intakes of folate/folic acid and the risk of various cancers have produced results which

cover a full spectrum ranging from significant benefits to significant increased risk, but most have produced equivocal or null results. Observational studies must now deal with a range of folate/folic acid intake from dietary sources alone to a mix of dietary folate and folic acid from supplements and fortified foods, with the latter changing over time. The matter is complicated by voluntary fortification of specific foods for market advantage which actually predates mandatory fortification of grains. Intervention studies that included high folic acid intakes have generally employed 1 to 5 mg/d which alone will produce high serum levels of UMFA. The connection between folate/folic acid and cancer is complex as is indicated by the well known effect of folate to promote cell proliferation and in particular the use of anti-folates such as methotrexate as chemotherapeutic agents.

Breast Cancer

Two large meta-analyses have recently been reported, both of which examined studies involving dietary only folate or dietary plus supplemental folate/folic acid.¹⁰⁻¹¹ No association with breast cancer risk was found. However, a recent study from Sweden of women in the Malmö Diet and Cancer Cohort found a protective effect but only when the lowest vs. the highest quintile of intake was compared.¹² In this study only 19% took supplements and the cut-point for the highest quintile was 349 µg/d. One of the first indications that high folate/folic acid might be harmful came from the Prostate, Lung, Colorectal and Ovarian Cancer Screening trial (PLCO). A 20% increase in the risk of developing breast cancer was found for postmenopausal women taking ≥ 400 µg/d of supplemental folic acid compared to those reporting no supplemental intake.¹³ The mean intake from supplements in the top quintile was 738 µg/d and the grand total in this quintile from food (after

fortification) and supplements was over 1200 µg/d. Consistent with all prospective studies,¹⁴ no statistically significant association was found between breast cancer risk and dietary folate intake. The mean intake in the top quintile from food was only 473 µg/d. Ulrich, in an editorial comment on the Malmö study, points out that the Swedish cohort had quite low dietary folate intakes and that it may be that it is in such populations that an inverse association between dietary folate and breast cancer is most likely to be observed. She also points to the evidence from the PLCO trial and suggests that the risk vs. folate/folic acid intake curve may be U-shaped, that women with adequate folate status derive no further benefit from additional intake, and that high folate status attributable to supplement use may increase the risk.⁹

A study from the UK found for women using folic acid supplements before and during pregnancy that 5 mg/d increased all-cause cancer death by 70% when the reference was a placebo or 200 µg/d, but the increase in breast cancer mortality was suggestive but not statistically significant.¹⁵ Also, it is well known that there is an interaction between alcohol and folate and in women with moderate alcohol consumption, taking 400 µg/d of folic acid results in the elimination of most of the alcohol-associated risk of breast cancer—another incentive for supplementation, but probably not common knowledge among the general public.

Colorectal Cancer

The recent report from the Polyp Prevention Study Group by Cole et al¹⁶ presents data indicating that folic acid supplementation might increase the risk of colorectal neoplasms, i.e. aggressive supplementation might enhance the growth of established microscopic lesions. This was a randomized clinical trial where all the participants had a prior his-

tory of excised colorectal adenomas. The endpoint was at least one new adenoma. Date on new neoplasms were collected with two colonoscopies conducted over a period of 6-7 years.

This study employed 1 mg/d of folic acid. Individuals taking multivitamins were not excluded (38% in the placebo group, 34% in the intervention group) and the period of recruitment coincided with the start of mandatory grain fortification in the USA. The percentage taking additional folic acid supplements over and above what was in their multivitamins was about 7% during the first follow-up interval, and increased to 14-18% during the second period. Thus some subjects could have had additional intakes of 400-800 µg/d of folic acid from multivitamins, and an unknown amount from folic acid supplements, and 200-400 µg/d or even more from fortified food. Total intakes of 2 mg/d might have been common, and amounts over 2 mg/d possible. Thus in this intervention study it is highly likely that all the participants had high levels of unmetabolized serum folic acid and some may have had very high levels. The authors admit that they carried out their study in a folate-replete population. It was found that the 1 mg/day dose of folic acid was associated with elevated risk of having 3 or more new adenomas when comparison was with the placebo group. In addition, the folic acid intervention was associated with an unadjusted increase in risk of 67% of developing at least one advanced lesion. Cole et al comment that their results are consistent with a recent observational study¹⁷ that found that adenoma risk is inversely associated with plasma folate levels only among individuals not taking multivitamins. Similar results were found in a study of colorectal cancer risk in women where dietary intakes of folate were significantly inversely associated with risk only among those not taking supplements containing

folic acid.¹⁸ As Kim points out in a recent review, the overall evidence supports an inverse association between folate status and colorectal cancer risk, but that folate appears to possess a dual modulatory effect on colorectal carcinogenesis that depends on the timing and dose of the intervention. For the progression of established neoplasms folate deficiency appears to have an inhibitory effect whereas folic acid supplementation appears to a promoter.¹⁹ This view was also expressed in an editorial by Ulrich and Potter.²⁰

There is also evidence of added complexity due to gene-nutrient interactions. A recent study by Vogel et al examined the role of folate in sporadic colorectal cancer associated with mutations in the adenomatous polyposis coli (APC) gene. The results suggested that folate enhances colorectal carcinogenesis through a distinct APC mutated pathway.²¹

Finally, it has been pointed out by Mason et al that there is a temporal association between the advent of folic acid fortification and an increase in colorectal cancer rates. They point out that both the USA and Canada have experienced abrupt reversals in the downward trend of colorectal cancer incidence that both countries had enjoyed in the preceding decade and that these changes do not appear to be explained by changes in the rate of endoscopic procedures. The reversal in both countries coincided with the beginning of mandatory grain fortification.²²

In all of these studies the potential role that UMFA might play is ignored even though in some cases the observed adverse effects could be due to this substance rather than elevated levels of folate itself. To give separate consideration to UMFA would obviously complicate most studies, especially given the already very complicated biochemical-genetic system in operation. In fact, it is normal for studies to lump folic acid and folate together in serum assays. This is, however not an

argument for ignoring the potential harm that could arise from high circulating levels of a molecule foreign to human biochemistry.

Prostate Cancer

Studies that examined the connection between prostate cancer and dietary folate/folic acid have been in general inconclusive. However, a study by Lawson et al adds to the concern about high doses of folic acid.²³ This was the NIH-AARP Diet and Health study which involved almost 300,000 men with a follow-up of 6 years. While no association was found between multivitamin use and the risk of localized prostate cancer, an increased risk of advanced and fatal cancers was found among men reporting multivitamin use more than 7 times per week. However, for men who took multivitamins more than 7 times per week and in addition took a folic acid supplement, significant enhanced risk was found for all prostate cancers considered together and for localized cancer.

The Connection between UMFA and Natural Killer Cell Cytotoxicity

Early in 2006 a paper by Troen et al appeared which may well have a very significant impact on the eventual understanding of the possible cancer promoting effect of UMFA.²⁴ In this study it was found that among a group of postmenopausal women, those who consumed a folate-rich diet and in addition used supplements containing > 400 µg/d of folic acid exhibited reduced natural killer (NK) cell cytotoxicity with a dose dependent lower cytotoxicity at higher levels of UMFA. UMFA was present in 78% of the women in the study group. The authors point out that the measured plasma folic acid was lower in this study than in other studies such as that of Kelly et al.¹ Thus even larger suppressions of NK cell cytotoxicity might be present in the

studies discussed above where the intake of folic acid was much higher.

Experimental and clinical evidence supports the role of NK cells in tumour cell destruction and they can be considered the first-line host defence against carcinogenesis.^{25,26} Thus there appears to be justified concern about UMFA. A decrease in NK cell cytotoxicity might contribute to the increased risk of colorectal adenomas observed by Cole et al²⁴ since there is a high probability that most if not all the participants had high levels of UMFA, given that the study dose alone would have been sufficient. The same would apply to the breast cancer study by Stoltzenberg-Solomon et al.²⁷ Troen et al point out that there is no clear mechanistic explanation for their observations and they regard their results as raising concerns about the independent toxicity of high levels of folic acid. It can only be hoped the study of Troen et al stimulates research into the many important questions raised. The suggestion that studies are needed of UMFA in the context of cancer is just now starting to appear in the literature.¹⁹

The Homocysteine Lowering Studies

Once it was generally recognized that elevated blood levels of homocysteine represented a significant risk for cardiovascular disease, there was great interest in attempting to reduce this risk by lowering the levels with supplementation using either folic acid alone or a combination of folic acid and either vitamin B₆ or B₁₂ or both. A number of studies have reported and Bazzano et al have provided a meta-analysis.²⁸ The folic acid doses ranged from 0.5 to 5 mg/d, with most in the range of 2.5 to 5.0 mg/d. The duration of intervention ranged from 6 to 60 months and all involved secondary prevention in patients with CHD, stroke or end-stage renal disease. Percentage reductions of homocysteine levels were

typically between 25 and 50%. The results have been uniformly disappointing with almost all studies yielding only a huge collection of statistically insignificant results for a variety of endpoints such as cardiovascular disease, coronary heart disease, stroke and all-cause mortality.²⁸ While these studies provide no direct evidence of adverse effects associated with high levels of UMFA in the context of the above endpoints, these high levels surely must have existed given the very high doses of folic acid used. It has been argued that the homocysteine lowering might have indeed had a beneficial cardiovascular effect, but that the high folate/folic acid levels had adverse effects which resulted in null results.²⁹ This is highly speculative and there do not appear to be convincing underlying biological mechanisms that might account for the proposed effect. UMFA does not appear to have been considered in this context, just high levels of "folate."

The impact of folic acid supplementation on in-stent restenosis after coronary artery stenting has also been investigated. Daily doses of 1.2 mg of folic acid were used along with vitamin B₆ and B₁₂. In a comparison to the placebo group, those receiving folic acid supplementation had a higher restenosis rate and a higher percentage requiring repeated target-vessel revascularization. After 250 days of treatment, major adverse coronary events were significantly more prevalent in the folic acid compared to the placebo group.³⁰ The mechanism appears unknown.

Cancer was a secondary endpoint in two large studies involving homocysteine lowering, NORVIT³¹ and HOPE 2.³² In the former, a 22% increase in cancer rate was observed for 0.8 mg/day of folic acid and 0.4 mg/d of vitamin B₁₂, but this result was only suggestive since it was not statistically significant. In HOPE 2 a dose of 2.5 mg/d of folic acid was used in combination with B₆ and B₁₂. Increased

risks of colon, lung, breast and prostate cancer were observed, but again, while the increased risks ranged from 11% to 36%, none achieved statistical significance although the 36% increased risk for colon cancer came close. These studies were not powered to produce statistically significant information regarding enhanced cancer risk if it existed and the number of cases was low, but the results are suggestive.

Cognitive Impairment and Folic Acid Fortification

It is well known that vitamin B₁₂ deficiency can produce profound cognitive impairment and that it is an ongoing challenge for clinicians to successfully accomplish differential diagnosis since in many cases this deficiency is easily treated, something that can not be said for other causes of cognitive impairment. Mandatory folic acid fortification has always come under scrutiny because it can mask vitamin B₁₂ deficiency with obvious serious consequences. Thus the study of Morris et al which found that high intake of folate may be associated with cognitive decline and anemia in older persons is of particular interest.³³ This cognitive impairment seen with high serum folate was only observed in individuals with low vitamin B₁₂ status. No information was available regarding the levels of UMFA since it was included in the total folate. Circulating UMFA has been suggested to play a role in either delaying diagnosis of vitamin B₁₂ deficiency by curing anemia, the masking phenomenon, or causing central nervous system problems.^{33,34} However, what is really going on is not well understood but clearly complex. As A. D. Smith points out in an accompanying editorial, there are a number of questions about the interaction of folate/folic acid and B₁₂ that need to be addressed as well as questions regarding food fortification and supplement use in the elderly popula-

tion, questions which include the merits of combining vitamin B₁₂ with folic acid fortification.³⁵

This recent study by Morris et al was consistent with an earlier study based on subjects from the Chicago Health and Aging Project where it was found that higher intakes of folate/folic acid were associated with cognitive decline in older persons.³⁶ In this study, the top quintile of total folate/folic acid intake had a range of 557-1719 µg/d and for those in this quintile, 96.9% used multivitamins.

Conclusions

While it might be argued that therapeutic use of folic acid, including daily doses of 1 mg/d or higher has been common for some time without evidence of adverse effects and therefore must be safe, such applications have not generally been in settings where side effects that develop slowly would be an issue or even noticed. Some patients may have had progressing co-morbidities, and especially in older populations the normal incidence of cancer would be hard to distinguish from an enhanced risk and would in general go unnoticed unless clinicians were especially alerted to the possibility. Most intervention studies using large or very large doses were neither looking for nor powered to detect increases in cancer risk. Those that investigated this question as a secondary endpoint detected enhanced risk. Thus it is not surprising that most of the evidence for risk associated with high dose folic acid has appeared in epidemiologic or intervention studies directed at primary or secondary cancer prevention.

Folic acid is a synthetic molecule which is foreign to human biochemistry. High levels of circulating UMFA are unique in human history. Trace amounts found in nature do not appear to invalidate this view. While it is true that folic acid can be converted into metabolites identical to those derived from dietary folates,

it appears that different pathways are involved and there remains the fact that at high folic acid intakes a metabolic saturation phenomenon exists which results in prolonged exposure to circulating unmetabolized folic acid, the consequences of which have not been investigated and remain largely unknown even though a decade has passed since Kelly et al¹ first raised the issue in a high profile journal. The observation of decreased NK cell cytotoxicity associated with high UMFA levels appears to be most significant since it provides one biological mechanism for adverse effects. But there may be a number of other mechanisms given the complexity of folate metabolism in general and in addition, the complexity of the gene-nutrient interactions associated with folate-mediated one-carbon processes.³⁷

This seems to be an area in urgent need of study, especially since there is growing interest worldwide in folic acid grain fortification and in some countries, ever increasing interest in supplements, and it has become possible to inadvertently consume large amounts of folic acid, especially if fortified ready-to-eat breakfast cereals and nutrition bars are eaten along with what is considered to be a common and safe multivitamin. It is not unreasonable to expect additional voluntary fortification with large amounts of folic acid in a number of prepared foods simply because of the promotional and marketing value associated with this action.

While few would argue with the manifest benefits and success of food fortification in the context of preventing neural tube defects, those benefiting represent a rather small fraction of the total population experiencing enhanced intakes of synthetic folic acid. If a portion of this larger population is indeed put at higher risk for various cancers, mental problems and possibly other as yet to be identified risks associated with high folic acid intake and the consequent high

levels of UMFA, then perhaps the food fortification program as well as voluntary fortification should be re-examined with this in mind. Also, consideration should be perhaps given to lowering the folic acid content of multivitamins if it is indeed true that many are getting 800 µg/d via this route alone. Especially significant in this context is the surprising result that higher levels of folic acid do not reduce the risk of cardiovascular disease even though there is a significant reduction in serum homocysteine. This removes an argument for fortification and for therapeutic doses. Finally, the concerns regarding B₁₂ masking have turned up in a different guise in the observation that high levels of folate/folic acid are associated with cognitive decline in the elderly who have poor B₁₂ status.

Thus significant public health issues appear to arise in the context of high folic acid intake. It would appear that the wrong form of folate is used for supplementation purposes and therapy, but there is at this point no other option. Folic acid was presumably selected because it was cheap to make and had a long shelf-life whereas natural folate was unsatisfactory in this context. This choice was made a number of years ago. About a decade ago questions were raised about adverse effects associated with UMFA but largely ignored and it is unfortunate that UMFA is not even considered in most studies. Nor has it been the central subject of studies such as, for example, its possible role in the early stages of metastasis. Until further research clarifies the question of risks associated with UMFA, it should be recognized that a very small amount of folic acid supplementation, e.g. 200-400 µg/d added to a diet containing modest amounts of unfortified but folate-rich foods will bring individuals up to a folate level which may well be quite satisfactory and certainly at the high end of the folate/folic acid intake observed

for healthy subjects in many older, pre-fortification observational studies, while at the same time virtually eliminating circulating unmetabolized folic acid. A deliberate effort to eat folate-rich foods can also accomplish this same end without supplementation. Fortification was intended to add only about 200 µg/d of folic acid to the diet, an amount estimated to have a significant impact on neural tube defects.

This paper should not be viewed as an attack on supplements but rather a call for considering the chemical form and dose of one very important and popular supplement which is also used for therapeutic purposes.

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