Folate status and colorectal cancer risk: a 2016 update

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Abbreviations. CRC: colorectal cancer; DFE: dietary folate equivalent; MTHFR: methylenetetrahydrofolate reductase; NTD: neural tube defect

1 **Abstract**

2 The consensus of epidemiologic evidence indicates that an abundant intake of
3 foodstuffs rich in folate conveys protection against the development of colorectal
4 cancer, and perhaps some other common cancers as well. Pre-clinical models
5 substantiate that the relationship is a genuinely causal one. Pre-clinical models
6 have also lent mechanistic insights into the biochemical and molecular pathways
7 by which adequate folate exposure conveys these protective effects, although
8 human studies are beginning to establish the relevance of our mechanistic
9 understanding to human cancer biology. Enhancement of genetic stability
10 appears to be a major mechanism by which folate sufficiency protects against
11 carcinogenesis. To date, the *Wnt* signaling cascade has been the pathway most
12 examined in this regard. The relationship between folate exposure and
13 colorectal cancer risk is a complex one, in part because a number of extrinsic
14 and intrinsic factors act as effect modifiers. This review discusses how the intake
15 of the other three B-vitamins integral to the 1-carbon pathway acts as one such
16 effect modifier. In addition, two concepts that remain matters of considerable
17 debate are whether parental intake of folate impacts on subsequent cancer risk
18 in the offspring, and whether excessive intakes of folate may have a paradoxical
19 cancer-promoting effect: observations underlying these two concepts are
20 presented as well.

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25 **1.0 Introduction**

26 Numerous epidemiological studies have been conducted over the past 25 years
27 that have explored the relationship between consumption of the B-vitamin, folate,
28 and the risk of developing cancer, and the overwhelming consensus of
29 observations indicates that greater dietary intake conveys protection against the
30 development of certain common cancers. The evidence has been most
31 compelling for colorectal cancer (CRC), with lesser degrees of evidence to
32 support such a connection for cancers of the breast, lung, pancreas and others
33 (reviewed in Chen *et al*, 2010). Pre-clinical experiments in a variety of rodent
34 models of intestinal cancer have substantiated the conclusions of these clinical
35 studies and proven, at least within the context of animal models, a true causative
36 role for inadequate folate intake as an independent risk factor for CRC (reviewed
37 in Kim YI, 2003).

38 The weight of evidence is quite strong in this regard and therefore the
39 reigning opinion among investigators in this field is that habitually inadequate
40 folate consumption is a genuine independent risk factor for CRC. This
41 conclusion was reiterated by the largest prospective cohort study to examine this
42 issue to date, in which ~525,000 adults were followed for over eight years, and
43 individuals consuming the greatest quantity of total folate versus those
44 consuming the lowest were found to enjoy a 30% smaller risk of developing CRC
45 (hazard ratio=0.70; 95% CI: 0.58-0.84) (Gibson *et al*, 2011).

46 However, not every clinical study on this topic has concluded that higher
47 folate status conveys protection: a few notable studies have reported either a null
48 effect of folate status on CRC risk (Eussen *et al*, 2010) or, curiously, a protective
49 effect of low folate status (Lee *et al*, 2012; Gylling *et al*, 2014). The absence of a
50 protective effect of higher folate status in these studies has yet to be explained,
51 although it is probably not a coincidence that each study that has failed to
52 demonstrate a protective effect of higher folate status has utilized blood levels as
53 the metric of folate exposure rather than oral consumption of the vitamin. One
54 potential explanation for these discordant results is that the studies that have
55 used blood concentrations to assess folate exposure tend to encompass much
56 smaller cohorts than those that have used dietary intake, and therefore may lack
57 sufficient power to detect a beneficial effect of folate. However, even in the
58 context of a single study where both measures of folate exposure were examined
59 there was a null association between serum folate and CRC risk but a quite
60 robust protective effect with increasing levels of dietary intake (Glynn *et al*, 1996).
61 This raises the question whether established modifiers of serum folate
62 concentrations such as smoking (Pfeiffer *et al*, 2015) and obesity (Bird *et al*,
63 2015) might complicate and obscure its relationship to CRC. Further, the serum
64 concentration of folate reflects the consumption of both natural sources of folate
65 as well as the pharmaceutical form of the vitamin, folic acid. This may be of
66 importance since at least one meta-analysis of prospective cohort studies
67 concluded that only natural sources of folate, and not folic acid, convey a
68 protective effect against CRC (Sanjoaquin *et al*, 2005).

Several other interesting facets of the relationship between folate exposure and the risk of CRC have emerged, and three are discussed in this review: 1) the modifying effects of the other 1-carbon vitamins, i.e.: B2, B6, and B12; 2) the possibility of a 'transgenerational effect', in which maternal intake of folate and other B vitamins during the periconceptional period, pregnancy and lactation might impact on the offspring's likelihood of developing cancer later in life. Indeed, there is even speculation that preconceptional paternal intake of folate might impact on spermatogenesis in a manner that modulates cancer risk in offspring, and 3) the concept of the so-called 'dual effect of folate' on cancer risk, whereby both inadequate as well as excessive intake of folate increase cancer risk when compared to adequate intake. The modulation of CRC risk associated with various polymorphisms in genes that encode enzymes that play roles in 1-carbon metabolism, especially the C677T single nucleotide polymorphism (SNP) in methylenetetrahydrofolate reductase (MTHFR), has recently been reviewed (Figueiredo *et al*, 2013) and will therefore not be discussed in depth in this review.

2.0 Scientific Background

As mentioned above, the consensus of over 30 case-control and prospective cohort studies indicate that individuals who habitually consume the greatest quantities of dietary folate have a 20-60% reduction in the risk of developing colorectal cancer as well as a similar reduction in the risk of having the precursor lesion, the adenomatous polyp (Chen *et al*, 2010). Moreover, multiple studies

92 indicate that the protective effect is more pronounced among those who are
93 moderate-to-heavy consumers of alcohol (Giovannucci, 2004; Gibson *et al*,
94 2011), a known inhibitor of folate metabolism (Mason and Choi, 2005). Not
95 surprisingly, a meta-analysis of the prospective cohort studies has confirmed the
96 protective effect of higher folate intake against the development of colorectal
97 cancer (Sanjoaquin *et al*, 2005). Although one must draw conclusions regarding
98 dosage cautiously from epidemiologic studies, the largest study to date would
99 suggest that ~500 micrograms of total folate per day conveys the maximal
100 protection (Gibson *et al*, 2011). The latter study did not report folate
101 consumption in terms of dietary folate equivalents (DFEs), which corrects for the
102 differential in bioavailability between folic acid and folate derived from food, so for
103 now the apparent optimal amount is necessarily described in terms of total
104 micrograms of the various forms of folate.

105 Several lines of evidence support a true mechanistic role for folate in the
106 prevention of cancer. First, controlled studies in a number of animal models
107 have demonstrated a dose-dependent protective effect of folate (reviewed in Kim
108 YI, 2004). Second, the fact that homozygous carriers of the common C677T
109 polymorphism of methylenetetrahydrofolate reductase have repeatedly been
110 shown to enjoy substantial protection against CRC also supports a mechanistic
111 role for folate availability in carcinogenesis (reviewed in Figueiredo *et al*, 2013)
112 although, interestingly, the apparent protection conveyed by this SNP largely
113 disappears among those who have low markers of folate exposure (Kennedy *et*
114 *al*, 2011). Finally, the likelihood of a true cause and effect relationship between

inadequate folate intake and an increased risk of cancer is strongly supported by the biological plausibility of the concept since folate is a critical co-factor in both biological methylation and nucleotide synthesis (Ciappio and Mason, 2010), and aberrancies of each of these two processes are thought to be among the most common mechanisms leading to cancer.

In further support of the mechanistic role played by folate depletion in colorectal carcinogenesis, studies in rodent models have identified the colonic *Wnt* cascade as a cell signaling pathway that is upregulated by mild inadequacies in folate status, especially when present in conjunction with mild inadequacies of the other related 1-carbon vitamins (Liu *et al*, 2007; Liu *et al*, 2011). Diets mildly inadequate in folate, B2, B6, and B12 have been shown to produce DNA strand breaks in the *Apc* gene, a negative regulator of *Wnt* signaling and a four-fold increase in *in vivo* *Wnt* signaling accompanied by an increase in proliferation and inhibition of apoptosis (Liu *et al*, 2011). Although a basal level of *Wnt* signaling is necessary to sustain the very high rate of proliferation normally present in the colonic crypt, inappropriate overactivation of the pathway due to genetic or epigenetic aberrations is found in over 80% of human colorectal cancers and is considered one of the earliest 'gatekeeper events' that enables normal colonocytes to follow a pathway towards neoplastic transformation. Lesser evidence has also implicated aberrations in the *p53* pathway as possibly playing a role as well (Liu *et al*, 2008).

It is rather tempting to also invoke anomalies in the methylation of DNA, and other methylated biomolecules as a mechanism by which folate inadequacy

promotes carcinogenesis but accruing evidence suggests this not to be true. Even rather severe degrees of folate depletion do not predictably induce aberrant methylation in the exons or promoter regions of critical tumor suppressor genes (Sohn *et al*, 2003). Also, biochemical ‘rescue’ of the methylation cycle within folate metabolism has been shown to not concur with reductions in tumorigenesis (Knock *et al*, 2008), whereas enriching the activity of cytosolic serine hydroxymethyltransferase, which operates on the nucleotide synthetic side of the folate metabolism cycle, markedly reduces tumorigenesis (McFarlane *et al*, 2014). The possible exception is the effect of maternal folate intake on cancer risk in offspring, where evidence is emerging that aberrations in DNA methylation might convey the transgenerational effect on cancer risk (see below).

Five prospective, randomized intervention trials have been conducted among individuals with previously resected colonic adenomas (Cole *et al*, 2007; Paspatis and Karamanolis, 1994; Jaszewski *et al*, 2008; Logan *et al*, 2008; Wu *et al*, 2009) in order to determine whether daily administration of folic acid supplements, at doses varying from 0.5-5 mgs/day, prevents the appearance of subsequent adenomas (adenomas, especially of the ‘high-risk’ variety are considered valid intermediary biomarkers of CRC risk (Schatzkin, 2000). The two trials considered to be the weakest (due to either their small size [Paspatis and Karamanolis, 1994] or to a less-rigorous-than-desirable study design [Jaszewski *et al*, 2008]) each reported a significant protection against adenoma development with folic acid supplementation. However, the meta-analysis of the three trials whose designs are considered to be the most scientifically robust is probably

more informative and accurate (n=2632 subjects in total): this analysis found that folic acid supplementation did not convey protection against adenoma occurrence in the subject population as a whole, although among those whose underlying plasma folate was in the lowest quartile at the onset of intervention a marginally significant 20% decline in the relative risk of adenoma occurrence was enjoyed among those receiving folic acid (Figueiredo *et al*, 2011). This is consistent with our present understanding, which is that adequate amounts of folate intake are protective compared to inadequate intake, but supplementation of those who are already folate-replete conveys no additional protection against CRC.

The evidence implicating a protective role for adequate folate status in other cancers is not as compelling, in part because the issue has not yet been as extensively examined, and because there is not a consistency of results among the studies. Nevertheless, there are observations implicating a protective role for adequate folate intake in human cancers of the oropharynx, esophagus, stomach, pancreas, lungs, cervix, ovary, breast, neuroblastoma and leukemia (Larsson, *et al*, 2006; Lewis *et al*, 2006; Larsson *et al*, 2007).

3.0 One-carbon vitamins other than folate as determinants of cancer risk

Folate is not the sole dietary component that operates as a critical co-factor in the network of biochemical reactions that is typically referred to as '1-carbon metabolism'. Consequently, assessing the impact of diet on the cellular integrity of 1-carbon metabolism by measuring folate status alone is often misleading

184 because vitamins B2, B6, and B12 serve as critical co-factors for several pivotal
185 enzymes as well. Further, other dietary components such as methionine and
186 choline are important substrates in 1-carbon metabolism as well. Consequently,
187 biochemical functions performed by 1-carbon metabolism are often modified by
188 the availability by several of these dietary factors. For instance, the disposal of
189 homocysteine (which ultimately determines plasma homocysteine
190 concentrations) reflects a complex 3-way interaction between folate, B12, and B6
191 status (Selhub *et al*, 1996). Moreover, since riboflavin is a co-factor for MTHFR,
192 plasma riboflavin is also a determinant of plasma homocysteine among those
193 who carry the C677T MTHFR polymorphism (Hustad, *et al*, 2000). Mathematical
194 modeling of the known enzyme kinetics of 1-carbon metabolism reveals that
195 critical functions of the pathway, such as nucleotide synthesis, are a complex
196 function of the availability of all four of these vitamins (Reed *et al*, 2006). The
197 inter-connectedness of these four vitamins is similarly important to those
198 metabolic outcomes that are relevant to carcinogenesis: for example, limiting B12
199 availability alone in a rodent model alters uracil incorporation in colonic DNA, a
200 function of 1-carbon metabolism that is 'distant' from the methionine synthase
201 reaction that B12 participates in (Choi *et al*, 2004). In addition, rodent studies
202 demonstrate that many of the pro-transformational molecular events that occur
203 as a result of altered 1-carbon metabolism, such as upregulation of *Wnt*
204 signaling, are greatly magnified when inadequacies of multiple 1-carbon
205 micronutrients are present compared to folate depletion alone (Liu *et al*, 2007;
206 Liu *et al*, 2011). A thorough mechanistic understanding of how folate modulates

carcinogenesis should therefore include an examination all four 1-carbon vitamins.

Clinical observations also provide a compelling argument for why all of the 1-carbon micronutrients should be examined in combination in regard to their impact on cancer risk. First, an increasing number of epidemiological studies have observed an association between low vitamin B6 and B12 status with an increased risk of common neoplasms (Lajous, *et al*, 2006; Zhang *et al*, 2003; Wu *et al*, 1999, Johansson *et al*, 2010), and these relationships persist even when the effects of folate blood levels or intake are regressed out of the relationship. Second, the study of these inadequacies is relevant to adult populations in the developed world: although flagrant deficiencies of these vitamins are uncommon in industrialized societies, marginal status, which is to say biochemical measures of vitamin status below the accepted normative range but in the absence of signs or symptoms of the classical deficiency syndromes, are rather common. Population-based studies have reported that 18-25% of adults have low B6 status and 10-20% of healthy elders are thought to have 'subclinical' B12 deficiency (Planells, *et al*, 2003; Lindenbaum, *et al*, 1994). Further, a recent nutrition survey of the United Kingdom reveals that >50% of adults have subnormal levels of the most accurate blood marker of riboflavin status (National Diet and Nutrition Survey, 2004).

4.0 Might maternal folate consumption impact on cancer risk in offspring?

Maternal nutrition is becoming increasingly recognized as a determinant of chronic disease in offspring (Barker, 2004), and this principle may apply to both macronutrients as well as select micronutrients. Although it remains speculative as to whether a genuine effect exists in the human population, there are a number of observations suggesting that a mother's habitual intake of folate pre-conceptionally, during pregnancy, and perinatally might impact on the risk of cancer in offspring. Epidemiological evidence does exist to support a protective role for high maternal folate intake against certain pediatric cancers in offspring, including retinoblastoma (French *et al*, 2003; Goh *et al*, 2007), non-Hodgkin's lymphoma (Schuz *et al*, 2007), acute lymphoblastic leukemia (Thompson *et al*, 2001) and childhood brain tumours (Goh *et al*, 2007), although, in balance, there is some inconsistency since some epidemiologic studies have not been able to observe such protection (Milne *et al*, 2010; Ajrouche *et al*, 2014; Chokkalingam *et al*, 2013; Orjuela *et al*, 2012). It is likely that the cellular pathways by which folate is thought to exert its impact on cancer risk in adults, as was described above, are also operable in the embryo, fetus and newborn. Moreover, although anomalies in DNA methylation due to folate inadequacy have not been convincingly shown to be instrumental in modulating cancer risk in adults (e.g. Sohn *et al*, 2003; Knock *et al*, 2008; McFarlane *et al*, 2014), the evolving fetus may be particularly susceptible to the establishment of aberrant patterns of methylation since marked swings in DNA methylation occur during embryogenesis (Li, 2002) and cells are dividing rapidly. Evidence that maternal folate intake can impact on the offspring's epigenome comes from studies with

252 'Agouti' (A^{vy}/a) mice in which maternal supplementation with folate and related
253 dietary methyl donors resulted in a shift in the offspring's coat color from yellow
254 to brown in conjunction with de-novo methylation of specific sequences in the
255 agouti gene (Cooney *et al*, 2002; Waterland and Jirtle, 2003) Also, maternal
256 plasma folate concentrations in humans were shown to concur with nearly 50
257 CpG sites in cord blood DNA, suggesting that a causal relationship between
258 maternal folate intake and the offspring epigenome may be relevant to human
259 biology as well (Joubert *et al*, 2016).

260 There are several studies in rodent models of colorectal and breast cancer
261 that support the concept that supplemental levels of folate intake in the mother
262 significantly diminishes the risk of cancer in her offspring. In a chemical
263 carcinogen model in rats, a comparison of maternal folic acid supplementation at
264 2.5 times the basal requirement versus the basal requirement prior to and during
265 pregnancy, and extending into the period of lactation diminished tumorigenesis in
266 offspring by two-thirds (Sie *et al*, 2011). Notably, a similar level of
267 supplementation provided to the pups after weaning did not convey protection.
268 Also, the fathers received identical diets to the mothers periconceptionally, so
269 protection conveyed by paternal diet can not be ruled out. Some differences in
270 the extent of genomic DNA methylation in the colorectum of the offspring were
271 noted between the diet groups, and this was suggested as a potential
272 mechanism by which the modulation of cancer incidence was effected. Similarly,
273 in a genetically-driven model of colorectal cancer in Apc1638N mice, maternal
274 supplementation of folic acid and the other 3 related B-vitamins prior to and

during pregnancy through weaning suppressed, while mild deficiencies promoted, tumorigenesis in the offspring (Ciappio *et al*, 2011). Suppression of the *Wnt* pathway in the intestines of the pups whose mothers received either the basal or supplemental levels of B-vitamins was suggested by changes in gene expression and β -catenin protein, providing a plausible mechanism by which maternal B vitamin intake might be modulating tumorigenesis in the offspring. There were also indications that changes in the gene expression of one of the negative regulators of *Wnt* signaling, *Sfrp1*, was being altered by changes in promoter methylation of that gene, providing further support to the theory that changes in DNA methylation might be a mechanism by which transgenerational transmission of risk is conveyed.

Considerably more speculative is the idea that paternal folate intake prior to conception might, to some extent, determine cancer risk in the next generation. In support of this idea are observations in mice that paternal folate depletion can lead to changes in the epigenome of sperm, and increased birth defects in the offspring (Lambrot *et al*, 2013). A single study comparing paternal B vitamin supplementation, repletion and depletion has been conducted in the *Apc1638N* model in which the endpoint of offspring intestinal tumorigenesis was examined. No overt changes in tumor incidence or multiplicity were observed although mean tumor volume increased in the female offspring in a stepwise fashion with increasing paternal B vitamin intake (Sabet *et al*, 2016).

Three rodent studies have examined whether maternal folate intake impacts on the risk of mammary cancer and the results are discordant. In one

study maternal folic acid supplementation compared to basal levels of the vitamin significantly reduced an intermediary biomarker of mammary cancer risk in female offspring, terminal end buds (Sie *et al*, 2009). Similarly, maternal supplementation of folate, B12, choline and methionine at five times that present in the basal diet reduced carcinogen-induced mammary carcinogenesis in female offspring (Cho *et al*, 2012). In contrast, maternal folic acid supplementation increased the risk of carcinogen-induced mammary tumors in the female offspring of rat dams (Ly *et al*, 2011). The inconsistency of results between these three studies makes it difficult to draw conclusions: perhaps the most important lesson to be learned is that different experimental protocols asking a similar research question can produce very different results, underscoring the need to determine which animal models are most relevant to human cancer biology and what details of a study protocol are the critical determinants of the outcomes.

5.0 The potential for cancer promotion with an overly-abundant intake of folate

Folate's central role as a co-factor in nucleotide synthesis means that abundant availability of the vitamin can facilitate the proliferation of rapidly dividing cells, and hyperproliferation is a feature of most dysplastic ('pre-cancerous') and malignant ('cancerous') neoplasms. The earliest evidence that folate consumption at levels achievable through the use of supplements might cause a paradoxical acceleration of carcinogenesis dates back to the 1940s when two

groups of investigators gave large doses of folic acid to patients with acute leukemia and observed what Sidney Farber politely termed “the acceleration phenomenon”, whereby the rate of expansion of the leukemic clone increased tremendously (Farber 1949; Heinle and Welch, 1948). The most intuitive explanation for this phenomenon is that neoplastic cells have a high rate of proliferation and therefore require large quantities of folate to maintain the synthesis of thymidylate and purines at the rapid pace required to meet heightened demands for DNA synthesis (Shpitz *et al*, 1997). However, other possible mechanisms exist. For example, supplemental folic acid has been reported to produce a transcriptomic environment that is pro-inflammatory in the human colon (Protiva *et al*, 2011), and chronic low-grade inflammation is thought to be a condition that pre-disposes to neoplastic transformation. Controlled studies in a variety of animal models of colon cancer support the contention that supplemental quantities of folic acid can promote tumorigenesis. In settings where there is a particularly strong underlying predisposition to colon cancer or in a setting where neoplastic tumors are already established, supplemental folic acid is protective only before neoplastic foci appear in the intestine. Once such foci are established, the more folic acid that is given, the faster microscopic foci and macroscopic tumors arise (Song *et al*, 2000a; Song *et al*, 2000b; Kim YI *et al*, 1996; Leleu *et al*, 2000; Wargovich *et al*, 1996).

The results of a randomized trial of folic acid in the prevention of colorectal adenomas among 1021 human subjects with a history of prior adenomas are consistent with such an effect (Cole *et al*, 2007). At the time of the second

344 follow-up, 6-8 years after initial randomization, this study observed a significant
345 2.3-fold increase in the multiplicity of recurrent adenomas among those receiving
346 folic acid supplements, as well as a marginally significant 1.7-fold increase in so-
347 called 'high risk' adenomas. One could reasonably speculate that a sizeable
348 number of individuals with high risk neoplasms could have been enrolled in this
349 trial in spite of undergoing a baseline 'cleansing' colonoscopy since the 'miss
350 rate' for advanced adenomas at the time of colonoscopy is thought to be ~10%
351 (Heresbach *et al*, 2008), and 'flat adenomas'--which are difficult to detect by
352 conventional colonoscopy--are common and much more likely to contain foci of
353 carcinoma than polypoid adenomas (Soetikno *et al*, 2008). If supplemental folic
354 acid in high quantities genuinely promotes the growth of indolent neoplasms, it
355 could then have accelerated the growth of these residual adenomas.

356 To date, four other folic acid supplementation trials that have used
357 adenoma recurrence as an endpoint have been reported (Paspatis and
358 Karamanolis, 1994; Jaszewski *et al*, 2008; Logan *et al*, 2008; Wu *et al*, 2009) and
359 none have observed a similar increase in tumorigenesis, but importantly, none of
360 these other four have had the extended follow-up over 6-8 years that was
361 provided for in the Cole *et al* trial. Similarly, when Ebbing *et al* combined the
362 results of two randomized homocysteine-lowering trials in Norway encompassing
363 over 6800 subjects, a 21% increase in the hazard ratio of developing cancer was
364 observed among those receiving folic acid supplements (Ebbing *et al*, 2009). It is
365 notable, however, that stratifying the data for individual cancers identified a
366 significant increase in lung cancer rather than colorectal cancer. In an

367 observational study, an increase of ~4 cases/100,000 individuals in the
368 nationwide rates of colorectal cancer diagnoses was reported to have
369 accompanied the first few years of mandatory folic acid fortification of cereal
370 grains in both the U.S.A. and Canada in the 1990s, and it was during the initial
371 years of these fortification programs when major millers were thought to have far
372 exceeded the mandated levels of fortification (Mason *et al*, 2007). Observations
373 from the abovementioned studies collectively raise a concern about the
374 possibility of a promotional effect of overly abundant intake of folate: 1) that is
375 consistent with the known biochemical functions of folate in DNA synthesis, 2)
376 that can be readily replicated in animal models of colorectal carcinogenesis, and
377 3) which is a phenomenon that has been observed to occur in both inadvertent
378 and intentional intervention trials in human subjects.

379 Moreover, organs other than the colon in which indolent foci of dysplasia
380 commonly exist in older individuals, such as the prostate, may be similarly
381 susceptible to this phenomenon. In the Cole *et al* trial, a secondary analysis of
382 prostate cancer incidence revealed that male subjects randomized to receive
383 folic acid supplements experienced a 2.6-fold increased incidence of clinically-
384 significant prostate cancer (Figueiredo *et al*, 2009). Similarly, in a meta-analysis
385 of prospective cohort studies, the odds ratio of developing prostate cancer was
386 1.18 ($p<0.02$) for every 10 nmol/L increase in plasma folate (Collin *et al*, 2010).
387 Also, in a meta-analysis of ten randomized, controlled trials in which folic acid
388 supplements were administered ($n>38,000$ subjects) the overall incidence of
389 cancer was increased to only a marginally significant degree ($RR=1.07$, 95% C.I.

1.00-1.14). However, when individual cancers were examined, the RR of prostate cancer was 1.24 (95% C.I.=1.03-1.49) (Wien *et al*, 2012).

The possibility of a paradoxical cancer-promoting effect of excessive quantities of folate may be operable in human breast cancer as well: in a prospective cohort study among ~25,000 postmenopausal women the risk of developing breast cancer was significantly increased among those taking folic acid supplements (Stolzenberg-Solomon *et al*, 2006); a group among whom consumption was ≥ 853 mcg of folate per day. Moreover, a promoting effect of folic acid has also been observed to occur in a rodent model of mammary carcinogenesis (Kotsopolous *et al*, 2005).

Nevertheless, the question as to whether excessive folate intake can promote cancer in some segments of human populations continues to be a very controversial topic since several null observations have been reported as well, arguing against such an effect. Clarke and his colleagues (Vollset *et al*, 2013) also performed a systematic analysis. This study included nearly 50,000 subjects enrolled in 13 RCTs in which the daily intervention was ≥ 500 mcg of folic acid. These investigators found a RR of cancer incidence of 1.06 (95% C.I. 0.99-1.13) associated with folic acid supplementation, and since the confidence interval bridged 1.00 the investigators concluded no significant effect on cancer incidence was likely. The data was also analyzed by the type of cancer, and after correction for multiple comparisons, no individual variety of cancer appeared to be increased by folic acid administration.

412 The results of two large, prospective cohort studies have also lacked
413 evidence of a promotional effect of excessive folate intake (Stevens *et al*, 2011;
414 Gibson *et al*, 2011). These two studies each reported a significant 20-30%
415 decrease in the risk of developing CRC among those taking the highest amounts
416 of total folate versus the lowest, with an apparent optimum for this purpose of
417 ~500 mcgs of folate from all sources per day. These two studies were large: in
418 the instance one the study population was ~100,000 individuals and in the
419 second, over 500,000. Nevertheless, as previously stated (Mason, 2011),
420 neither the Gibson *et al* (Gibson *et al*, 2011) nor the Stevens *et al* (Stevens *et al*,
421 2011) studies were able to satisfactorily examine those segments of their study
422 populations that would be most susceptible to a cancer-promoting effect of high
423 folate intake, such as those ingesting >1000 mcgs of total folate per day or those
424 harboring indolent foci of colonic or prostate neoplasia.

425 In this era it is not at all difficult to consume >1000 mcgs of total folate in a
426 day. By consuming a multivitamin and a bowl of fortified ready-to-eat cereal in
427 the morning, federally mandated fortified flour in various foodstuffs during the
428 course of the day, and a B-complex tablet at night, one can easily exceed 1300
429 µg of folic acid per day (and this does not even include the ~200-250 additional
430 micrograms ingested by most adults as naturally-occurring folates).

431 Re-stated, the 'dual effect' hypothesis contends that higher intakes of
432 folate are cancer-protective in nearly all circumstances except among those
433 individuals who have existing neoplastic lesions and who are consuming
434 exceptionally large quantities of the vitamin, in which case the folate presents

additional risk for tumorigenesis. Cancers that slowly evolve over years through an indolent dysplastic phase, such as the colorectal adenoma or a dysplastic nodule of the prostate, are exceedingly common in the general population of healthy, elder adults and would be particularly prone to such an effect.

The fact that folic acid and not a naturally-occurring form of the vitamin is used by the food and pharmaceutical industries for fortification and supplementation is potentially of importance. Folic acid is converted to a natural biological form of the vitamin as it passes through the intestinal wall, with enzymatic reduction and methylation resulting in the circulating form of the vitamin, 5-methyltetrahydrofolate. Nevertheless, oral doses of folic acid as small as 200 mcgs can saturate this conversion mechanism (Sweeney *et al*, 2003; Kelly *et al*, 1996; Sweeney *et al*, 2006; Sweeney *et al*, 2007), resulting in detectable levels of circulating folic acid, and it is theorized that this oxidized, non-substituted form of folate might feasibly be detrimental due to some idiosyncratic action that does not exist for naturally-occurring forms of folate, possibly because it can bypass certain regulatory nodes in the 1-carbon metabolic scheme, entering as THF rather than 5-methylTHF. In the U.S., 40% of adults 60 years and older are thought to regularly consume a supplement containing folic acid, most of which contain 400 mcg per pill (Radimer *et al*, 2004), and therefore it is not surprising that a nationally representative survey found that ~40% of individuals over 60 had detectable levels of unmetabolized folic acid in the blood (Bailey *et al*, 2010). Of additional concern are recent animal and clinical observations indicating that high folic acid exposure might

possess an idiosyncratic effect of inhibiting the cytotoxicity of circulating natural killer cells (Troen *et al*, 2006; Sawaengsri *et al*, 2016). Natural killer cells are a population of lymphocytes thought to play a role in the destruction of arising clones of neoplastic cells. Thus, the coenzymatic form of folate used for fortification is perhaps relevant in determining whether a detrimental effect is produced from excessive intake, although the concern remains a theoretical one. When examined in a nested case-control fashion, detectable levels of unmetabolized folic acid did not predict the occurrence of colorectal cancer (Cho *et al*, 2015).

The question of whether there is indeed a cancer-promoting effect of excessive folate intake in the human population remains a hotly debated issue. On the one hand, observations suggesting that a cancer-promoting effect has been created among segments our population are rather weak, but on the other hand the biological plausibility of the effect can not be denied and the large-scale ramifications of such an effect, if it exists, are not ones that we can afford to overlook.

6.0 Conclusions

In our opinion sufficient evidence has accrued to conclusively state that habitual intake of adequate quantities of folate, compared to inadequate intake, sizeably reduces the risk of developing colorectal cancer, and a similar effect may exist for other cancers as well. There are clearly several effect modifiers such as alcohol ingestion, intake of related B-vitamins, relevant genetic variants: these

are factors that need to be considered when examining this topic. The question of whether maternal (or even paternal) intake of folate might impact on cancer risk in the subsequent generation, as well as the issue of whether folate might act in a paradoxical, cancer-promoting fashion in certain segments of our society remain matters of open debate. A more in-depth understanding of these phenomena will be needed if we are to fully exploit these principles for the purposes of cancer prevention.

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