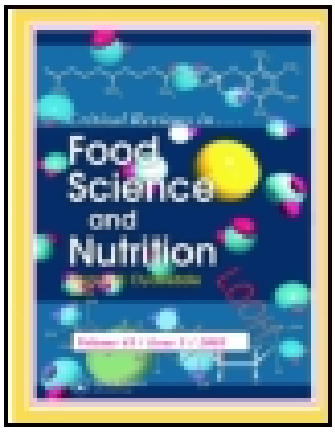

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Application of the Key Events Dose-Response Framework to Folate Metabolism

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Abstract

Folate is a vitamin that plays a role as a cofactor and coenzyme in many essential reactions. These reactions are interrelated and any change in folate homeostasis could affect other reactions. With food fortified with folic acid, and use of multivitamin, unmetabolized folic acid (UMFA) has been detected in blood circulation, particularly among older adults. This has raised concern about the potential harmful effect of high folic acid intake and UMFA on health conditions such as cognitive dysfunction and cancer. To examine what is known about folate metabolism and the release of circulating UMFA, the Key Events Dose-Response Framework (KEDRF) was used to review each of the major key events, dose-response characteristics and homeostatic mechanisms of folate metabolism. The intestine, liver and kidneys each play essential roles in regulating body folate homeostasis. But the determining event in folate metabolism leading to the release of UMFA in circulation appears to be the saturation of dihydrofolate reductase in the liver. However, at each of the key events in folate metabolism, limited information is available on threshold, homeostatic regulation and intracellular effects of folic acid. More studies are needed to fill in the knowledge gaps for quantitatively characterizing the dose-effect relationship especially in light of the call for extending folate fortification to other foods.

Keywords: folate, folic acid, food fortification, Key Events Dose-Response Framework, unmetabolized folic acid

Folate is a vitamin that plays a role as a cofactor and coenzyme in many essential reactions. These essential reactions include nucleic acid synthesis and numerous methyl transferase reactions such as DNA methylation and amino acid metabolism. These are all interrelated reactions and any change in folate homeostasis could affect other reactions (Figure 1).

Mandatory folic acid fortification of grain products implemented in 1998 in the U.S. has been effective in reducing neural tube defects (NTD) in newborns. However, studies show that among supplement users, intake may exceed the Tolerable Upper Intake Level (UL) of 1mg/day (Choumenkovitch et al., 2002 Quinlivan and Gregory, 2003). Also, the appearance of unmetabolized folic acid (UMFA) in blood circulation has been detected in about 40% of adults aged 60 years and older (Bailey et al., 2012, Morris et al., 2010). This has led to concern that UMFA may disturb cellular folate uptake and normal intracellular folate metabolism. To date it is unknown whether high intake of folic acid and the presence of circulating UMFA or high serum folate are responsible for adverse health outcomes but evidence from some studies suggest such possibility (Cole et al., 2007; Figueiredo et al., 2009; Morris et al., 2010; Troen et al., 2006).

A potential negative outcome of high folic acid intake may be its effect on cognitive impairment among vitamin B12 deficient older adults (Morris et al., 2007; Morris et al., 2010; Schneider et al., 2006). Another concern is that high folic acid intake may increase the risk of recurrence of colorectal adenoma (Cole et al., 2007). The concern that high intake of folic acid may lead to adverse outcomes makes it crucial to explore ways to identify the minimum effective intake of

folic acid that is required to prevent NTDs while minimizing the potential risk of negative outcomes due to excessive intake among vulnerable populations.

To review what is known about each of the major key events, dose-response characteristics and homeostatic mechanisms along the folate metabolism pathway we used the framework known as the Key Events Dose-Response Framework (KEDRF), a component of the International Life Science Instituteôs (ILSI) Global Threshold Project (Julien et al., 2009). This analytical approach is a useful tool in integrating knowledge and identifying research gaps. The KEDRF provides an organizing structure for the systematic analysis of individual key steps and considers the mechanism between intake and effect of concern and on the overall dose-response relationship for the effect of concern (Julien et al., 2009). Although it is currently unknown what the biological and physiological impact of UMFA is, its increased presence in blood circulation is a phenomenon that needs investigation and is a potential effect of concern. Therefore, using KEDRF, we review the major steps or key events in the metabolic pathway of folate leading to UMFA (Figure 2 and Figure 3). KEDRF has been applied in a case study with vitamin A and found to be useful in shedding light on the relationship between high vitamin intake and potential adverse effects (Ross et al., 2009). In this review, we identify control points which are the mechanisms that help maintain a normal physiological environment. The capacity of such mechanisms to keep homeostatic balance is likely to influence the overall dose-response relationship between total folate intake and UMFA. Certain control points may play an especially critical role in a given pathway; and if the outcome of these control points greatly influence the likelihood of the ultimate effect of concern, such as the appearance of serum UMFA; then such

control points are identified as determining events (Julien et al., 2009). In addition, we identify gaps in our knowledge of folate metabolism that would be necessary for the determination of a dose-response relationship for folate. We use folate as a collective term for naturally-occurring folate and fortified/supplemented folic acid; however, due to their different chemical structure, they may undergo different biochemical reactions in certain metabolic steps and thus will be discussed separately, when necessary.

Functions and adverse effects associated with folate

Folate functions as a coenzyme in single-carbon, methyl group transfers in the metabolism of nucleic acids and amino acids. Folate is crucial for normal DNA synthesis and is needed for pyrimidine and purine biosynthesis (Figure 1). Pyrimidine nucleotide biosynthesis requires folate co-enzyme in the conversion of deoxyuridylic acid to thymidylic acid (Kim, 2005). Folate is believed to have dual effects (promotion and inhibitory effects) on cancer development (Kim, 2004). One of the hypothesis is that a deficiency in folate may lead to an increase in cellular uracil to thymidine ratio, thus, misincorporating uracil into DNA, destabilizing DNA molecule and potentially leading to an increased risk of malignancy (Blount et al., 1997). Additionally, cytosine methylation altered by folate deficiency has been shown to up-regulate proto-oncogene expression and induce cancer (Duthie, 1999). Several prospective studies have shown an inverse association between folate intake and cancer such as; breast cancer (Zhang et al., 1999, Ericson et al., 2007), colon cancer (Fuchs et al., 2002, Giovannucci et al., 1998) and ovarian cancer (Kelemen et al., 2004, Larsson et al., 2004), especially among alcohol drinkers.

In contrast, results from limited studies showed a positive association between high folate intakes and cancer. Such an association between folate intake and colorectal cancer was hypothesized from the results of two ecological studies (Hirsch et al., 2009; Mason et al., 2007). However, results of epidemiological studies have been inconsistent (Rampersaud et al., 2002). Animal studies have also provided critical information concerning the dual modulatory effects of folate on the development and progression of colorectal cancer (Kim, 2004) and on the protection against colorectal cancer (Kim et al., 1996). Whether and how folate may exert these dual effects on colorectal carcinogenesis appears to depend on the timing and dose of the folate intervention (Ulrich, 2007; Ulrich and Potter, 2007). The mechanism lies in the essential role of folate in DNA synthesis and on the biological methylation reactions.

Either deficient or excessive folate could also be related to cognitive dysfunction in the presence of low vitamin B-12. Folate and vitamin B-12 are essential in the conversion of homocysteine to methionine, the precursor of S-adenosyl methionine (SAM). SAM is a methyl donor to a wide range of reactions involving DNA, protein and lipids. Folate acts as the methyl donor where 5-methyltetrahydrofolate (5-MTHF) is converted to tetrahydrofolate (THF) and the methyl group is donated to homocysteine to form methionine (Figure 1). The deficiency of folate and vitamin B12 is associated with reduced cellular SAM and elevated homocysteine levels (Refsum H., 1998; Robinson et al., 1998), potential disrupted formation of myelin (which is essential for the proper functioning of the nervous system) (Weir and Scott, 1999), as well as hindrance of DNA synthesis and cell division (Blount et al., 1997; Koury and Ponka, 2004).

Additionally, vitamin B_{12} is the only acceptor of the methyl group from 5-MTHF and homocysteine is the only acceptor of methyl-B_{12}. Thus, a deficiency in vitamin B_{12} can generate a large pool of methyl-THF that is unable to undergo reactions and will mimic folate deficiency and accumulate homocysteine. The only way for the 5-MTHF to be recycled to THF, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B_{12} - dependent enzyme methionine synthase. In its absence, cellular folate will become progressively trapped as 5-MTHF. The concern is that if folic acid is ingested, nucleotides may be synthesized and the hematological picture of megaloblastic anemia will be normalized but not the neurological symptoms associated with vitamin B12. It has been hypothesized that these changes may lead to masking of vitamin B12 deficiency and to the progression of potentially irreversible neurological symptoms (dementia, paresthesia and ataxia) (Cuskelly et al., 2007).

Uptake from lumen into enterocyte

Digestion in lumen

Most naturally occurring folates are polyglutamate derivatives. These are hydrolyzed to monoglutamates on the surface of intestinal mucosa by the hydrolase enzyme glutamate carboxypeptidase II (GCP II) prior to absorption into the intestinal cells. Folic acid is a monoglutamate, so hydrolysis is not necessary for its absorption.

GCP II is located on the human jejunum brush border and is sufficient to hydrolyze dietary folate intake (10 cm proximal jejunum in humans contains GCPII to hydrolyze 200 µg folate) (Reisenauer and Halsted, 1987). There is currently no evidence to show that excess intake of

folate will saturate GCP II. There appears to be adaptive up-regulation of the enzyme in case of folate deficiency (Said et al., 2000), but the molecular mechanism and threshold dose that would trigger this up-regulation has not been identified. It is unclear whether GCP II is down-regulated in the presence of excess folate.

Thus, GCP II is not an important control point, although it is adaptively regulated. Reisenauer et al. (Reisenauer et al., 1986) reported that the rate of hydrolysis of polyglutamyl folate by brush-border GCP II is more than 100-fold faster than the rate of monoglutamyl folate transportation into intestinal cells, which suggests that the transport of the monoglutamate end-product is the rate-limiting step in the process of dietary folate absorption.

Transportation into intestinal cells

Folate monoglutamates are hydrophilic anionic molecules and at physiologic concentrations of luminal folate (<10umol/L), the uptake mainly occurs via a carrier-mediated process (Rosenberg et al., 1985). In this process, two proteins; the Reduced Folate Carrier (RFC) and the Proton-Coupled Folate Transporter (PCFT), are responsible for intestinal folate uptake in the proximal jejunum and duodenum (Zhao et al., 2009). This process requires energy and is dependent on acidic pH. However, at high amounts of folic acid, passive diffusion through nonsaturable mechanism does occur (Rosenberg et al., 1985).

RFC is an integral membrane protein and is ubiquitously expressed in tissues to play a central role in tissue folate homeostasis (Sirotnak and Tolner, 1999). RFC has a high affinity for reduced folate and a low affinity for folic acid (Matherly and Goldman, 2003). PCFT prefers oxidized

folate such as folic acid to reduced folates as substrates, although affinities for all folate analogs for PCFT are high at micromolar range (Yuasa et al., 2009; Zhao et al., 2009). Both RFC and PCFT are inversely responsive to folate concentration, so that uptake is reduced when dietary folate intake is high and increased when intake is low (Liu et al., 2005; Said, 2004). In animal and in vitro studies, both mRNA and RFC protein expression increased in a folate deficient environment (Said et al., 2000; Subramanian et al., 2003). Similarly, PCFT mRNA levels increased about 13-fold in the proximal small intestine of mice that were fed a folate-deficient diet, as compared to those fed a folate-replete diet (Qiu et al., 2007).

Conversely, down-regulation of RFC and PCFT occurs under conditions of high folate supplementation. It has been reported that levels of 100 ε mol folic acid/L in culture media lead to significantly reduced folic acid uptake in Caco-2 cells, as well as a significant decrease in RFC and PCFT mRNA levels (Ashokkumar et al., 2007). Similarly, in a rat model, acute high supplementation of folic acid led to significant decrease in intestinal folate uptake by down-regulating the expressions of RFC and PCFT (Dev et al., 2011). The regulation of intestinal uptake appears to be mediated by transcriptional regulatory mechanisms. It is unclear what amounts of folate and folic acid in humans will saturate the carrier proteins leading to passive diffusion.

In summary, the carrier-mediated intestinal process is important for maintaining normal physiological folate levels and meeting metabolic requirements. Uptake from the lumen into the enterocyte is a homeostatically regulated event, and is a control point influencing folate

homeostasis. Folate deficiency and supplementation may lead to a respective up-regulation and down-regulation of folate transporter molecules, as evidenced by respective increases or decreases in their mRNA and protein levels. However, at high levels of folic acid intake, passive diffusion may occur and folic acid enters the cells. The specific mechanisms that dictate how folate levels affect the mRNA and protein levels of folate transporters are not clear. Also, the threshold for folate and folic acid absorption is unclear.

Intestinal intracellular metabolism and distribution

Folate monoglutamate and folic acid are transported into intestinal cells where intracellular metabolism and distribution occurs. Once these folate analogs are in enterocytes, intracellular metabolism such as reduction, methylation and folylpolyglutamate synthesis takes place. Some of the folic acid will undergo an additional reduction process to dihydrofolate (DHF) and THF catalyzed by dihydrofolate reductase (DHFR). THF will be methylated to 5-MTHF before entering the portal circulation (Darcy-Vrillon et al., 1988; Izak et al., 1972). Some of the 5-MTHF will be stored in cells by having glutamate residues added to the molecules to increase their size and hence prevent them from leaving the cells (Egan et al., 1995).

The capacity of DHFR to reduce folic acid in intestinal cells is low and some of the folic acid in its unmetabolized form will enter the portal circulation. Additionally, DHF is a potent inhibitor of methyl tetrahydrofolate reductase (MTHFR) (Matthews and Baugh, 1980); therefore, high concentrations of folic acid could potentially inhibit the formation of 5-MTHF and lead to a decrease in methionine and SAM (methyl donor) synthesis. In those with poor vitamin B-12

status, methionine synthesis is already compromised, so this mechanism could exacerbate the methyl group deficiency. To our knowledge, no data is available to indicate the amount of folic acid that can saturate DHFR in humans.

After intracellular modifications, monoglutamylfolates, mainly 5-MTHF and UMFA are transported through the enterocyte basolateral membrane into portal circulation. The efflux is transported via a carrier-mediated and active anion exchange mechanism (Said et al., 1987). Similar to the brush-border membrane, RFC and PCFT are located at the basolateral membrane responsible for trans-membrane transport (Said and Redha, 1987). However, compared with the brush-border membrane transport system, the transport capacity of RFC is lower at the basolateral membrane in the presence of intracellular neutral environment (Said et al., 1987; Said and Redha, 1987) and PCFT is believed to be the major carrier at this level (Qiu et al., 2006).

In this key event, monoglutamate and folic acid are biotransformed or leave the cells intact. The DHFR which catalyzes the reduction of folic acid is a control point. DHFR has low capacity and so folic acid may exit the cells for the portal circulation in its unmetabolized form.

Hepatic metabolism and distribution

Enterohepatic circulation of folate

Liver is the major site for folate storage and processing. Folate is taken up from the portal circulation and enters the liver in the form of monoglutamate (THF and primarily 5-MTHF) and UMFA. The transport process is saturable energy-dependent at low folate concentration, while at

higher concentration of folate (up to 20 µmol/L), the uptake is not saturable (Horne et al., 1989). Once taken up by liver, more than 97% of 5-MTHF is rapidly cleared into bile. While 15-20% of THF is retained in liver predominately in the form of polyglutamate for storage (Steinberg et al., 1979). The liver contains half of the bodyôs folate. Though a substantial fraction of the folate is shunted to bile, most of the folate in bile is reabsorbed in the intestine via enterohepatic circulation (Shin et al., 1995).

Folate metabolism in liver

Monoglutamate and folic acid are taken up by the liver primarily by PCFT. Folic acid undergoes conversion to its biologically active form via reduction, methylation and polyglutamate formation (Zhao et al., 2009). Similarly to what occurs in the enterocyte, DHFR is also the enzyme responsible for reduction of folic acid in the hepatocyte, and its activity is a major control point in the hepatic metabolism of folic acid. Compared to rats, human DHFR activity is quite low (Whitehead et al., 1987). It takes about an hour to convert 400 ε g of folic acid to the reduced form, while the time to convert 5 mg could take up to 12 hours (Bailey and Ayling, 2009). The limited reduction capacity of DHFR results in the appearance of UMFA in plasma and urine. Bailey and Ayling (2009) reported that a concentration of about 331 ε g of folic acid in human liver could saturate available DHFR. They suggested that human liver is the fundamental cause for the appearance of UMFA in blood circulation. The authors reported that DHFR activity is low and quite variable in human samples and suggested that the plasma concentration of UMFA and possibly DHF will vary between individuals according to their DHFR activity.

Therefore, this control point which may become saturated by excessive intake of folic acid is a determining event as it does not fully control the release of UMFA in blood circulation.

The appearance of UMFA in serum may also be affected by genetic polymorphism. DHFR polymorphism which includes the deletion of a specific 19-bp region of DHFR gene was associated with higher expression of DHFR (Parle-McDermott et al., 2007; Xu et al., 2007). This polymorphism was implicated in increased risk of breast cancer among supplement users. It was suggested that higher enzyme activity may lead to greater one carbon metabolism in favor of DNA synthesis and at the expense of methyl supply (Xu et al., 2007). This polymorphism may also be associated with a decreased risk of neural tube defect (Parle-McDermott et al., 2007). In contrast, data from the Framingham Offspring Study showed that individuals with DHFR polymorphism had higher circulating UMFA (Kalmbach et al., 2008b). Further studies are needed to clarify the role of this polymorphism in humans.

Effort was made to determine the threshold at which UMFA starts to appear in blood circulation. Kelly and colleagues reported that oral folic acid intakes of 266 εg/meal resulted in UMFA in serum, while no UMFA was observed in serum of subjects taking less than 200εg/meal (Kelly et al., 1997). Similarly, Sweeney et al. detected UMFA in serum of folic acid replete individuals after an oral dose of 200 εg per meal administered twice a day for 7 days (Sweeney et al., 2007). The increased appearance of serum folic acid after the second 200 εg per meal dose of folic acid indicate that accumulation may occur over time. In the Framingham Offspring Study serum folic acid was measured before and after mandatory folic acid fortification and the results show that

exposure to fortification significantly increased circulating concentrations of folic acid, total plasma folate, and 5MTHF among both supplement and non-supplement users (Kalmbach et al., 2008a).

In summary, in this key event, most of the 5-MTHF is circulated back to the intestine via enterohepatic circulation. Folic acid is reduced by DHFR, however, that enzyme has low activity and, intake of 200 ε g /meal may lead to the appearance of UMFA in circulation. Thus this event- conversion of folic acid by DHFR to its reduced form- may be responsible for the appearance of UMFA in blood circulation and, is considered a determining event.

Kidney folate reabsorbtion

Folate is transported in blood primarily as monoglutamate in three forms: free folate, folate bound to folate binding protein and folate bound to albumin, all of which can be filtered by human kidney (Selhub et al., 1987).

Filtered folates are reabsorbed back to circulation from the proximal tubule cells and folates not reabsorbed are finally excreted in urine. Therefore, the homeostatic level of folate in human body is regulated by the reabsorption of filtered folate in the proximal tubule cells. The reaborption is mediated mainly by three types of receptors; folate receptors (FR), megalin and cubilin, which are all located in the kidney proximal tubule epithelial cells. Of these receptors, FR is the most important one and has high affinity for a number of folate compounds including folic acid (Damaraju et al., 2008). The expression of FR is regulated by extracellular folate concentration;

however, results of studies are somewhat contradictory depending on experimental model used. In vitro studies show that FR expression on renal epithelial cells was down-regulated with over-supplementation of folic acid at a level of 100 µmol/L culture medium (Ashokkumar et al., 2007), and up-regulated under low-folate conditions (Henderson et al., 1988; Kane et al., 1988). In contrast, a low-folate diet led to down-regulation of FRs in studies on mice and rats (da Costa et al., 2000; Gates et al., 1996). This down-regulation is suspected to occur due to proteolysis of the membrane anchor for the receptors which become unsaturated as a consequence of the diminished folate concentration in the glomerular filtrate (76). Once folate is taken up by the receptors, the reabsorbed filtered folate crosses the basolateral membrane to blood circulation primarily via RFC which is located on the basolateral membrane of kidney tubules (Wang et al., 2001).

Renal tubular reabsorption plays a pivotal role in maintaining folate homeostasis by reabsorbing the filtered folate. However, the regulation of tubular folate uptake remains to be established under both excess and deficient folate and folic acid intake.

Tissue uptake, storage and intracellular metabolism of folate

Folate uptake at peripheral tissues occurs via folate receptor FR and membrane carrier RFC (Zhao et al., 2009) and these are overexpressed in the presence of folate deficiency (Backus et al., 2000; Gates et al., 1996; Jansen et al., 1990; Zhu et al., 2002). Of concern, is that UMFA could theoretically compete with 5MTHF for carrier protein and binding protein with special

concern for folate metabolism in the brain (Smith et al., 2008). This mechanism, however, has yet to be demonstrated.

Once monoglutamates enter mammalian cells they are rapidly modified for storage by the addition of several glutamate residues to form long side chains in order to trap the folates within the cells. Polyglutamate synthetase plays a regulatory role in maintaining a relatively constant tissue folate concentration by increasing the polyglutamate chain length in periods of folate deficiency (Nijhout et al., 2004). It is suggested that modest increases in cellular concentrations of folate may activate the folate-dependent reactions while large increases may inhibit those reactions and their related enzymes (Nijhout et al., 2004; Smith et al., 2008).

Little is known about intracellular effects of folic acid. Some hypotheses suggest that folic acid might disrupt folate metabolism by disturbing the balance of folate and several folate-dependent enzymes, where elevated folic acid could act as inhibitor. The accumulation of DHF, the first step in folic acid metabolism, may inhibit purine and thymidine synthase and consequently interfere with DNA synthesis (Allegra et al., 1985; Dolnick and Cheng, 1978). Additionally, high folic acid also may inhibit SAM (methyl donor) synthesis by inhibiting MTHFR (Matthews and Baugh, 1980) and decreasing methylation reactions.

One of the theories put forward is that excess folic acid among individuals with low vitamin B12 may allow cell division in bone marrow to proceed, as it is independent of methionine synthesis, but these growing cells could place increased demand for methyl group further depleting

available methyl groups and worsening the impact on non-proliferating cells of the nervous system (Scott and Weir, 1998; Smith et al., 2008).

Tissue uptake and storage of folate is a key event of folate metabolic pathway. However, knowledge of the regulatory mechanisms within cells is limited and at this time several theories have been put forward to describe potential impact of excess folic acid on health outcome, however, these have yet to be determined.

Discussion

The Key Events Dose-Response Framework developed by ILSI, is an interesting and flexible analytical approach to reviewing nutrient metabolism because it can be used to integrate research findings within key events and channel this information towards an endpoint of interest. In this paper we use the release of UMFA in blood circulation as our endpoint. There is no evidence that folic acid in circulation occurs naturally, so the appearance of folic acid in the blood indicates that excess folic acid intake has occurred due to supplement use and/or fortified food.

Folate is regulated within each of the key events. The intestine, liver and kidneys each play essential roles in regulating body folate homeostasis. At each of these key events, however, limited information is available on folate threshold and homeostatic regulation. Additionally, little is known about intracellular effects of folic acid. The determining event in folate metabolism leading to the release of UMFA in circulation appears to be the reduction of folic acid to DHF and THF by the liver enzyme DHFR. Excessive intake of folic acid can saturate DHFR. Once the saturation threshold is reached in the liver, UMFA is released into circulation.

Results of studies indicate that the lowest amount of folic acid intake that resulted in serum UMFA is an oral dose of 200 εg. There is also some indication that folic acid accumulation occurs over time. It is currently not uncommon to detect UMFA in blood circulation, particularly, among older adults especially since folic acid is present in supplement and fortified food. Intakes exceeding the Tolerable Upper Intake Level (UL) of 1mg/day has been reported (Choumenkovitch et al., 2002; Quinlivan and Gregory, 2003) and its impact must be evaluated especially among vulnerable populations such as children and older adults.

Epidemiological studies have shown conflicting results in the association between UMFA and negative health outcomes such as cognitive dysfunction and cancer. Several theories were put forward to explain the mechanism by which UMFA may lead to negative outcomes. One theory states that high folic acid supply DHF and THF providing substrate to proliferating cells at the expense of methyl reactions. The impact of these changes may include masking of potentially irreversible symptoms of cognitive dysfunction and providing substrate to cancer cells. Alternatively, studies have shown that increase in total cellular folate may inhibit folate-dependent reactions. Therefore, the question still remains as to whether UMFA, increase in total cellular folate or both, exert adverse effect and how.

Results of studies indicate that the appearance of UMFA in serum varies by individuals. This may be due to genetic makeup and possibly to the presence of DHFR polymorphism. Further studies are needed to clarify the role of this polymorphism in humans and to identify other potential genetic effects on folate metabolism as a consequence of excessive folate intake.

Limited knowledge of the regulatory mechanisms and the threshold for some key events limit our ability to quantitatively characterize the dose-effect relationship and yet it is critical to do so at this time in light of the advocacy by some agencies to extend food fortification to corn masa flour, a basic ingredient in many foods, such as corn tortillas, that are predominantly, but not exclusively, consumed by the Hispanic population. Compared to other race-ethnicities, Hispanic women have higher rates of infants born with NTDs and lower total folic acid intake (Hamner et al., 2009). However, an increase in the food supply, must be evaluated carefully to avoid negative impact to vulnerable populations especially Hispanic older adults.

Finally, folate is involved in many reactions that could affect DNA synthesis and amino acid metabolism. Any change such as deficiency or excess could potentially affect a cascade of responses. It would, therefore, be of interest to apply KEDRF to some of the other endpoints in folate metabolism to examine the impact of excess intake on other pathways.

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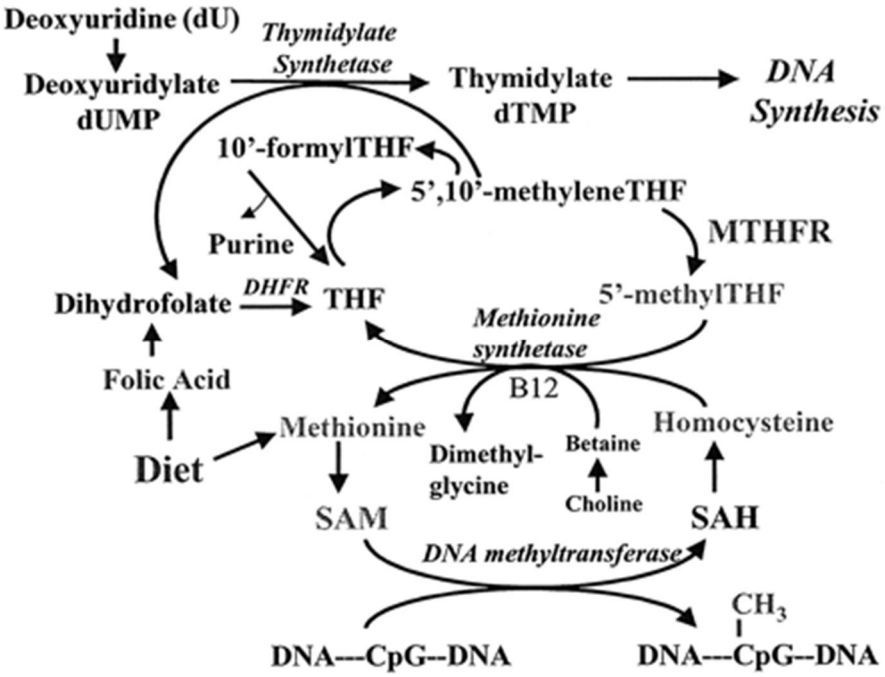

B12, vitamin B-12; DHFR, dihydrofolate reductase; CH3, methyl group; CpG cytosine-guanine dinucleotide sequence; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate.

Source: Kim, 2005

Figure 1. The role of folate and vitamin B12 cofactors in the methylation cycle

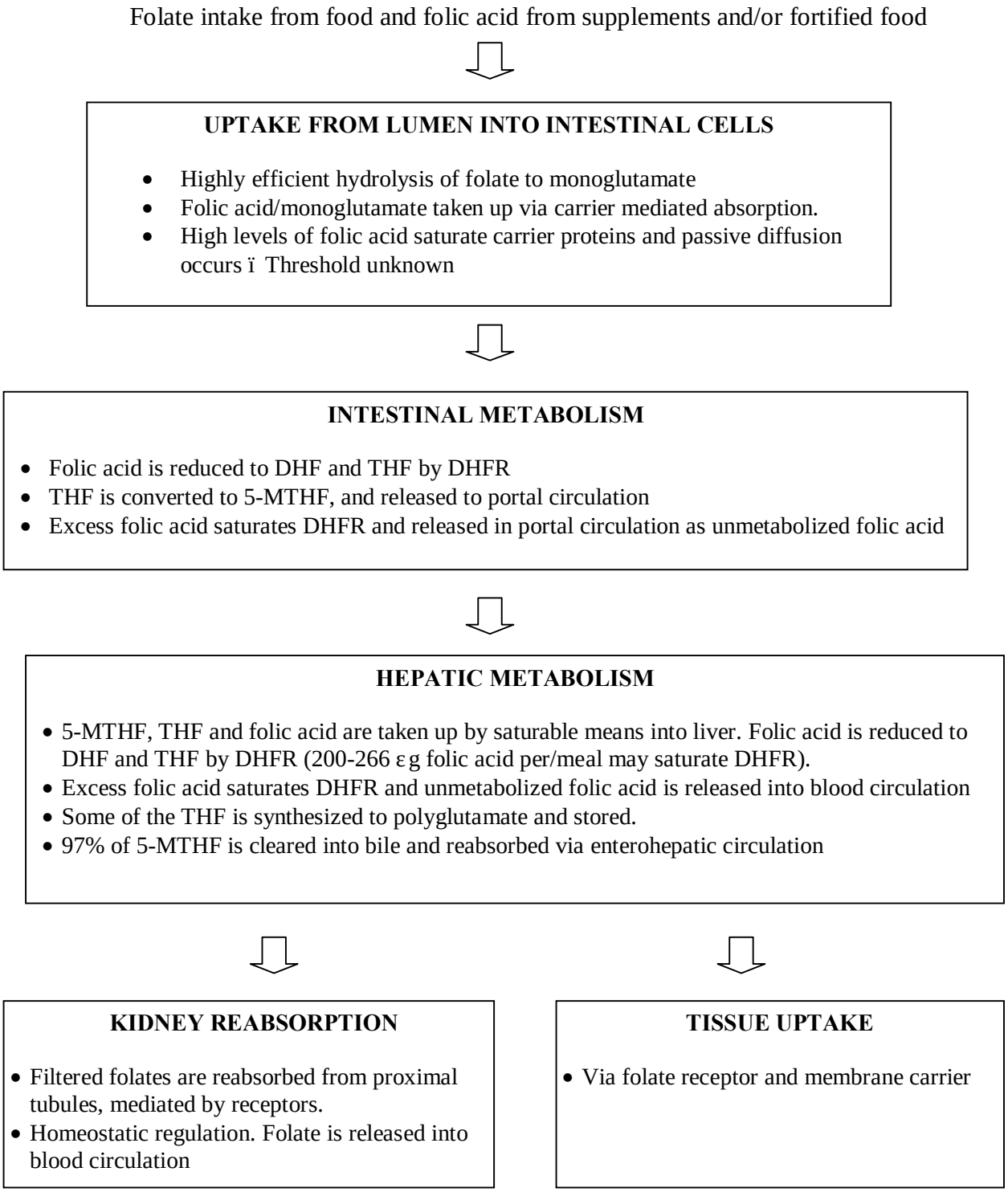

Figure 2. Key Events and Control Points in the Metabolic Pathway of Folate

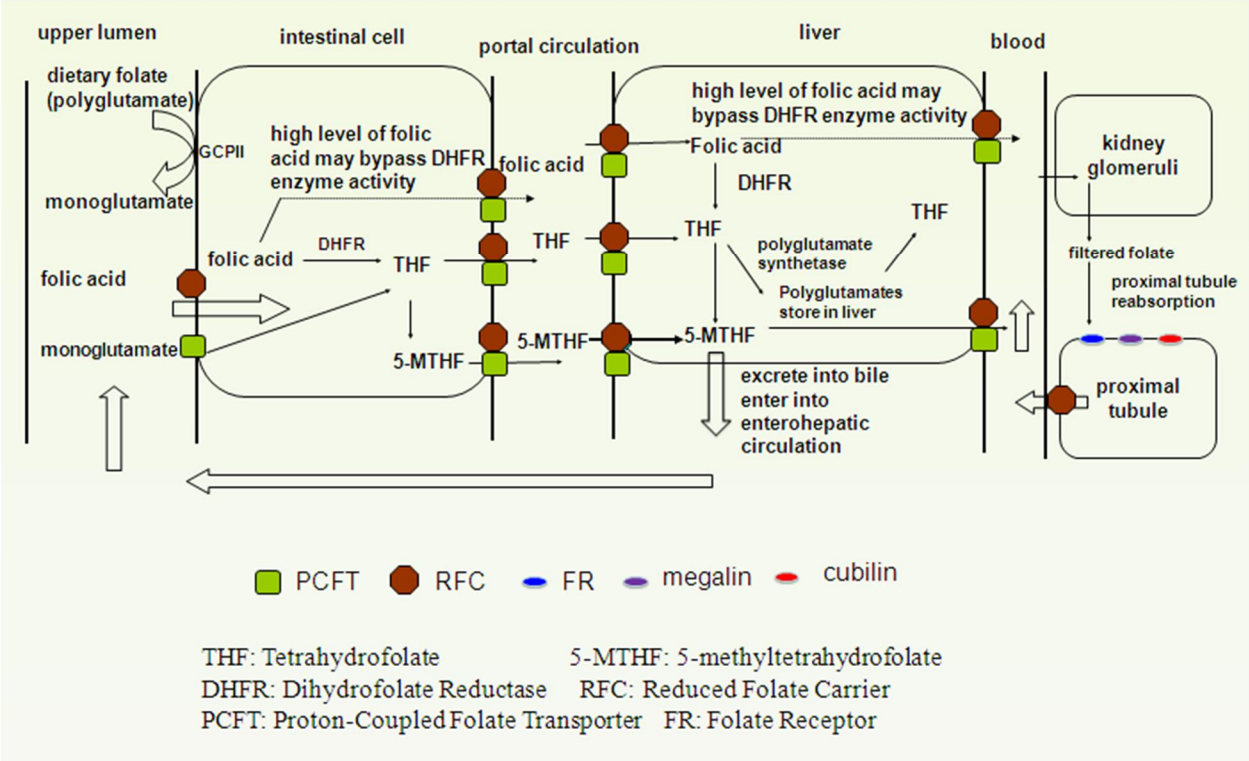

Figure 3. Folate Metabolism Pathway