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Folates: Key Nutrients to Remember

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Abstract

Folic acid or vitamin B9 or pteroylglutamic acid, is a relatively simple molecule with two characteristics; firstly, it must be reduced by 2 or 4 hydrogen atoms to be metabolically active which makes it sensitive to oxidation and must be protected by ascorbic acid, secondly it may include in addition to the constituent residues of the molecule, 1-7 glutamate residue at one of its ends. These polyglutamate forms that make up the largest share of food folate, must be deconjugated by a specific enzyme present in the intestinal lumen before being absorbed in the jejunum. It is in the methylated form after passing through the enterocyte it is transported in the blood, excreted in bile and reabsorbed. It must be demethylated to integrate folic cell cycle and methyl transfer, that allows the synthesis of methionine (only possible in the presence of vitamin B12), purine, serine and especially thymidylic acid, constitutive DNA. As a methyl donor that plays a fundamental role in cerebral and nervous metabolism. Folates are involved in cell division thus; any folic acid deficiency causes a slowdown in rapid multiplication systems which may lead to red blood cell disorders (macrocytic anemia), immunity, and neural tube defects, in addition to physiological disorders (cardiovascular, cancer ...). Folic acid supplementation appears to allow the correction of these disorders.

Keywords: Deficiency, Disorders, Folic acid, Supplementation

1. Introduction

Folic acid is a vitamin found between 1931 and 1939[1] in liver and yeast as a factor whose absence caused macrocytic anaemia in rats and growth arrest of *Lactobacillus casei*[2,3]. In 1941, Folic Acid (or F.A.) was isolated and received its name from spinach (folium meaning leaf in Latin). The term "folates" includes different forms of folic acid. In 1943, it was successfully isolated by Stokstad and was synthesized by Angier et *al.* in 1946[4] and Taylor and Hanna in 1975[5]. As all vitamins, F.A. is essential to the organism which is unable to synthesize it. F.A. is abundant in green vegetables, seeds (almonds, corn, chick peas ...) and liver[6,7]. Severe folate deficiency can be observed in malnourished pregnant women, particularly in developing countries, where socio-economic conditions are unfavourable[8,9]. By its key role in the synthesis of DNA, this vitamin is essential in any cellular division, whatever it is and especially in high-growth organisms, as are the embryo and the foetus and in rapid renewal tissues as hematopoietic organs, the digestive mucosa or the immune system. Folic acid deficiency can also affect the nervous system function and the behaviour[10–13].

2. Folate Metabolism

2.1 Folic Acid

Folic acid is a quite simple molecule composed of pteridine nucleus linked to Para-Amino-Benzoic Acid (PABA) and Monoglutamate (MG); on which is articulated a chain of glutamic acid giving polyglutamate of folic acid or Polypteroylglutamates, more or less hydrogenated and charged with a one-carbon group (methyl, formyl, methenyl, Methylene) on their pteroic part[14–17] (Figure 1).

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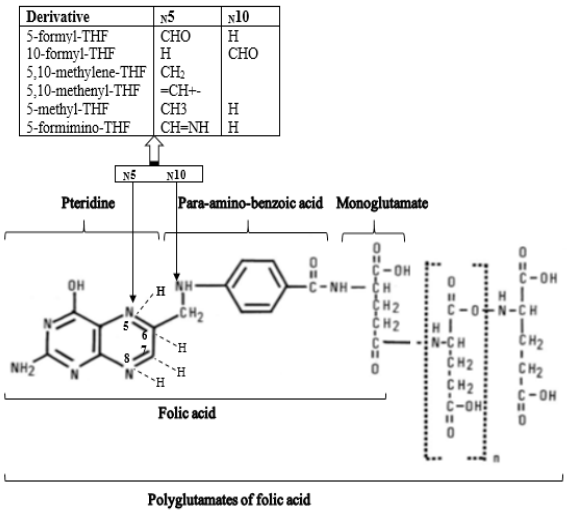

Figure 1. Chemical structure of folic acid and its derivatives.

Folic acid or PGA is practically no present as such in nature where it exists only as traces in human tissues and foods, it represents the purified, stable form used in pharmaceutical preparations most of which is as Polyglutamates (PG)[15,18-21]. Folic acid is a yellow, water-soluble crystalline powder that is sensitive to air, light and heat; this explains the losses observed during cooking[22].

2.2 Absorption

The vitamin is absorbed in the proximal portion of the small intestine by an active process[23], but all along the small intestine through a passive mechanism[24–26].

2.3 Mechanism

Most of folates are present in foods, in the reduced form or bound to proteins, as methylated or formulated polyglutamate[27,28]. The polyglutamate forms are converted to monoglutamate derivatives (MG) by action of digestive proteases[21,29]. Only MG forms crossed the enterocyte's barrier as confirmed by several works in animals[30,31], in humans[14,29,32,33] and in vitro[15,34]. The folate conjugase responsible for this hydrolysis, also called pteroyl polyglutamates hydrolase or polyglutamate hydrolase, has been detected in many tissues and medias: intestine[35,36], blood, bile, pancreas, kidney[37-38], brain[31] and liver[30]. Within the enterocytes, the Tetrahydrofolic Acid (THF) is produced from dihydrofolic acid by folate reductases and then converted into 5 methyl tetrahydrofolates which is the only form joining the portal circulation[19,31,39] (Figure 2).

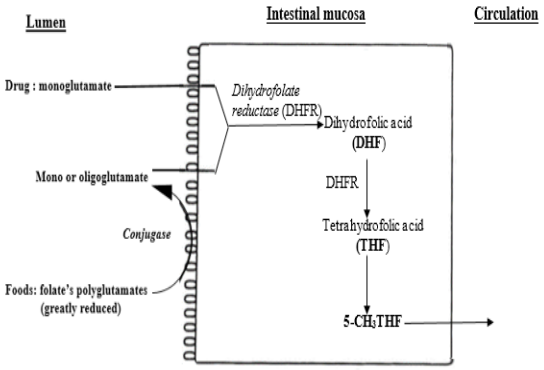

Figure 2. Intestinal absorption of folates.

2.4 Transport and intracellular entry

Transport is the limiting step in the absorption of MG and PG[15,17,34]. Furthermore, specific proteins with high affinity are involved in transport of folate called "Folate Binding Proteins" (FBP) found in: milk[40,41], placenta[41], serum[42,43] and urine[44].The absorbed methyl tetrahydrofolate is anchored on cells surface on specific receptor protein[45]. The folate-protein complex enters in cell where folic derivative is released and the receptors can then be re-expressed on the cell surface. The transmembrane transport of folates is regulated by intracellular folate levels[29].

2.5 Role of Folic Acid and Main Metabolic Pathways

In order to be metabolically active, folic acid is reduced either partially by folate reductase to dihydrofolate or completely by dihydrofolate reductase to tetrahydrofolate (THF)[46]. Dihydrofolate reductase is inhibited by antifolates such as methotrexate and aminopterin[47], pyrimethamine[48]. THF function as coenzyme in the transport of one-carbon units (methyl, methylene, formyl, formimino). Interconversions of folate derivatives are catalyzed by several enzymes whose steps are presented in Figure 3.

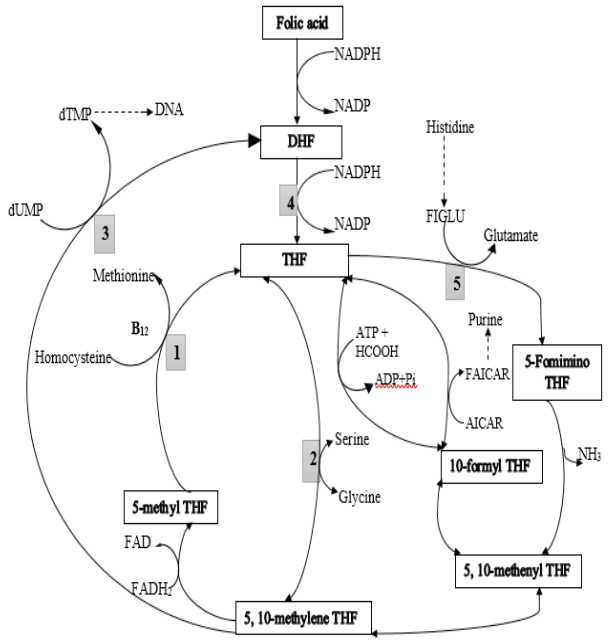

Figure 3. A simplified representation of folate interconversion cycle.

1: methionine synthetase, **2:** transhydroxymethylase serine, **3:** thymidylate synthetase, **4: d**ihydrofolate reductase, **5:** formiminotransferase, **DHF:** dihydrofolate, **THF:** tetrahydrofolate, **dUMP:** deoxyuridine monophosphate, **dTMP:** deoxythymidine monophosphate, **FIGLU:** formiminoglutamate, **(F) AICAR:** 5 (formyl) aminoimidazole carboxamide ribonucleotide.

2.6 Storage

Folates are found in all body tissues and media. The liver is the main organ of storage, the average rate of hepatic folate is 10 pg/g tissue[8,19]. The bile folate rate is very high; it is 10 times the serum. Hepatic folate is partially excreted in the bile and reabsorbed[19]. The enterohepatic cycle aims to tissue redistribution. It should be noted that red cell folate represents an important part of the reserves folate (1 ml of packed red cells contains 0.25 µg of folate).

2.7 Elimination

It is estimated that only 0.3 to 0.8% of the folate pool is excreted daily[50]. Urinary excretion is low (1 to 10 pg/d)[51] while tubular reabsorption is important. 15 times the amount of ingested folate are contained in faeces[52]. This is explained by the existence of an important folic synthesis by the intestinal flora (60 to 90 µg/d) but not available to host[53] because it is localized beyond of the intake pool.

3. Nutritional Aspects

3.1 Naturel Sources of Folates

Folates are found in wide variety of food; very abundant in leafy vegetables, such as spinach, salads ... but also in soy and wheat germs, brewer's yeast, egg yolk, fermented cheese, seeds (corn, chickpeas and almonds). The levels of folate cow's milk and human milk are relatively low (50 µg/l) and very low in goat's milk[54]. Even if the bioavailability of folates arises good, folates may be destroyed by cooking food mainly with water and in divided doses (up to 80%)[7].

3.2 Folic Acid Requirements

The minimum requirement of folates for an adult is 70 pg/d, to maintain the biological values constant, the body requires 150 to 200 pg/d[55]. Demands for folates increase during pregnancy, lactation and growth[56,57], as is the case for smoking, intestinal disorders, infections and traumas, chronic haemolysis, anti-epileptic or anti-cancer treatments, hyperthyroidism, oral contraception and the HIV virus[58].

3.3 Folate Status of Populations and Manifestations of Folates Deficiency

In 1960, Herbert and his collaborators[59] conducted the first dosages of folates in the blood which were mainly used in the differential diagnosis of megaloblastic anemia due to deficiencies of vitamin B12 or folate. But the problem quickly became apparent to detect early deficiencies in folate, without anemia. The evolution of the onset of clinical symptoms found by Herbert are only valid for assessing severe deficiencies, they do not solve the problems of detecting early or marginal deficiencies[60]. The comparison of biochemical status and dietary intakes of folate reached a good degree of correlation through the current food composition table[61]. Assessment of serum or plasma folates, erythrocyte or blood cell folate levels is most commonly used for the monitoring of folates status[62]. However, the assay methods of these compounds are not standardized which poses problems in the comparison of the data[63]. The value of red blood cell folate is considered the best indicator of folate status; it

represents the state of liver reserves. A value less than 100 µg/l and a VGM greater than 100 µm^3 in a group reflects a situation with a high risk of deficiency, a value superior to 200 µg/l, the optimum zone is reached. Between 100 and 150 µg/l, there is a risk but moderate, which can be more or less severe depending on the individual differences[7]. Serum folate are very influenced by the amount of folate absorbed in the recent period, unless we practice three successive measurements[64].

3.3.1 Evolution of Folate Status Indicators in Deficiency Situation

In 1962, Herbert[60] conducted experiments on volunteers, including himself, to a diet very poor in folates providing 5 to 10 µg AF per day, was able to show the different phases that succeed at the haematological and biochemical level[66] (Figure 4).

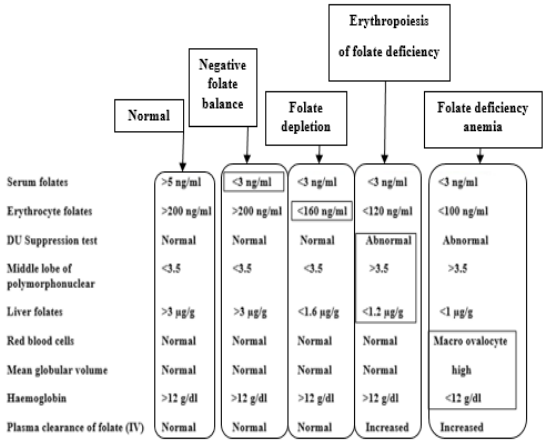

Figure 4. Experimental folate deficiency in humans: Sequence of biochemical and haematological abnormalities[66].

3.3.2 Haematological Manifestations

Folates deficiency is associated with a defect in the synthesis of nucleic acids which hinders cell division affecting mostly erythrocytes. Folate deficiency in an advanced stage is responsible for deep macrocytic anaemia, sometimes with leukopenia and thrombocytopenia. The polymorphonuclear cells are hypersegmented and there is a megaloblastic deficiency[65].

3.3.3 Neurological and Neuropsychiatric Disorders

Later, lead to behavioral and psychiatric disorders, anxiety, memory problems, fatigue, insomnia[60,67–69]. Congenital disorders (metabolic diseases), they are manifested by mental retardation and motor disorders[6,11,12]. Folate deficiency has been associated with hyperhomocysteinemia and neurodegenerative diseases, including Alzheimer's and Parkinson[70–72]. Type the information in a paragraph/another picture.

3.3.4 Digestive Disorders

Dyspeptic disorders, constipation or diarrhea[11].

3.3.5 Immune Disorders

Recent research has shown that folate deficiency can lead to abnormalities of the defense system against infections[73,74].

3.3.6 Risk of Cancer

Numerous studies have related folate's status and the appearance of several types of cancers (Cervix, colorectal, lung, esophagus, brain, pancreas, breast ...)[75]. It has been shown that several aspects of one-carbon metabolism are involved in the increased risks of developing cancer[76]. A lack of thymidine in case of folate deficiency can lead to abnormal incorporation of uracil during the synthesis of DNA and DNA repair defects. This increases the frequency of DNA mutations and thus the risk of developing cancers[77,78].

3.3.7 Cardiovascular Diseases

Folate deficiency is also considered as an independent risk factor of cardiovascular diseases affecting coronary or peripheral territories. It has been associated with a high concentration of total homocysteine with an increased risk of formation of atherosclerotic plaques in the aorta[79], risk of stroke[80], myocardial infarction, ischemic heart disease or arrhythmia[81]. Homocysteinemia in healthy subjects was also correlated with other cardiovascular risk factors such as total cholesterol and apolipoproteins A and B[82].

3.4 Etiology of Folate Deficiency

Outside of pregnancy, the most frequent aetiologies of folate deficiency are:

Inadequate food intake by loss of appetite in older people and alcoholics[83,84], low socio-economic group, malabsorptions such as celiac disease, tropical sprue,

surgical procedures in the digestive tract and intraluminal microbial outbreaks. The defects in use are drugs such as methotrexate used in chemotherapy[47]. Others have secondary "folate-depressant" effects; this is the case for oral contraceptives, anti-epileptics[85] and even aspirin[29], other vitamin deficiencies mainly the vitamin B12 deficiency and vitamin C.

4. Folic Acid in Pregnant Women

The risks of deficiency were mainly expressed in pregnant women for whom the needs are greatly increased due to the expansion of maternal tissues: uterine growth, placenta construction, foetal development (intensive cellular multiplication of the foetus and constitution of its reserves).

4.1 Causes and Effects of Folate Deficiency in Pregnant Women

The possible deficiency's causes can be of three orders: lack of input, increased needs and increased losses.

Lack of input: Due either to the overall decrease in ingestates if vomiting is frequent in early pregnancy or inadequate intake of foods rich in folates. To this last cause may be added culinary habits favoring the prolonged and especially fractionated cooking of the food, even rich in folates.

Increased needs: the increased need for folate is linked on the one hand to the physiology of erythropoiesis[86] and on the other hand to the "folate pump" that represents the foetus for its development and the constitution of its reserves. Folate requirements increase more postpartum[87] because of the consequent loss during lactation[88].

Increased losses: losses can be increased by an important urinary excretion and can be evaluated to 15 µg/d instead of 9 pg/d[89]. This is due to hormonal impregnation of pregnancy[90].

4.2 Particular Consequences of Folate Deficiency during Pregnancy

The effects of folate deficiency of the pregnant woman can reach both the mother and the child.

4.2.1 In the Mother

Deep folate deficiency may be responsible for megaloblastic anemia. Among pregnant women in developing countries, there is a 60% megaloblastic marrow prevalence, and even in industrialized countries, 25-30%[65,91] with or without macrocytosis or anemia. Some authors have reported on pregnant women deficient in folate: Preterm deliveries, repeated abortions, in which retroplacental haematomas are frequent[92].

4.2.2 In Children

The mother having folate deficiency may have babies not only with low birth weight[93,94] but also congenital malformations especially defective closure of the neural tube, three anomalies can occur: Anencephaly, encephalocele and the most important spina bifida[95,96] and the genetic abnormalities of cerebral development: trisomy 21, X-fragile[97,98].

4.3 Need for Folic acid Supplementation during Pregnancy

All these manifestations of folate deficiency justify the fact that systematic folate supplementation is recommended in pregnant women and in new-borns with low weight at term or premature.

4.3.1 Prevention of Folic Acid Deficiency and Megaloblastic Anemia

Lowenstein et *al.*[99], Edelstein et *al.*[100] and Fleming et *al.*[101] observed that folate supplementation resulted in a reduction in the incidence of megaloblastic anemia during and after pregnancy.

4.3.2 Reduction Incidence of Malformations

The works carried out by Laurence[102] et *al.*[103] and Smithells et *al.*[104], Mulinare et *al.*[105] and MRC[96] permitted first to advance the hypotheses of the role of folate deficiency in the determinism of certain fetal abnormalities, such as cleft palate and neural tube malformations of the child carrying spina bifida which is an abnormality of the closure of the neural gutter in the first weeks of the uterine life. A supplementation with AF, with or without other vitamins and minerals, administered before another pregnancy to women who have already given birth to affected children showed that the risk of recurrence of the abnormality is decreased from 5% to 0,5%[104] and it lowered from 70% in another large study, conducted on more than 1000 women by the MRC[96].

Identical findings on prevention of recurrences of labial fissures were reported by Tolarova in 1982[106]. A recent study suggests that periconceptional folic acid supplementation was associated with a lower risk of autistic disorder; these findings do not allow us to establish a cause and effect link, they need to be confirmed[107].

4.3.3 Fortification

In 1998 the United States and Canada made mandatory the addition of 140 µg of folic acid per 100 g of cereal products (15 µg/100 g for Canada). This policy has been established by the US Food and Drug Administration[108] to prevent neural tube defects (NTD) but this fortification, such affected in the United States, appears to be difficult to implement particularly in developing countries. The fortification program has known very successful; the incidence of NTD in the United States decreased in a range of 19% -27%[109], the number of NDT passed from 4000 in the period 1995-96 to 3000 in 1999-2000[110]. The undesirable effect most know of supplementation and food fortification with folic acid is the masking the diagnosis of B12 deficiency, because megaloblastic anemia caused by cobalamin deficiency may be reversed, but not the long-term potential of neurological effects[111]. In the United States, all women of childbearing age are advised to have a daily intake of folic acid of 0.4 mg/d: with folic acid fortified general food products (cereals) for of reducing their risk of having an infant affected with spina bifida or other NTDs[109].

5. Conclusion

Recent epidemiological studies have shown a preventive effect of folic acid on physiological disorders and on neural tube defects in children, this sparked a renewed interest in this vitamin, which few Algerian scientists had hitherto concerned. Finally, a genetic sensitivity can be corrected by nutritional factor.

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