Nutrient-Gene Interaction: Folic Acid and Prenatal Neural Tube Defects

Author: Christe Marbbn

April 2, 2009

Key words: Folic Acid; Neural Tube Defects; Homocysteine; Methionine; *S*-adenosylmethionine; Prenatal Development

Nutrients play a major role in influencing gene expression and protein interaction. The effect of dietary changes on phenotypes differs significantly between individuals. Some individuals appear to be relatively insensitive to dietary interventions, while others are highly sensitive (Roche, 2006). This phenomenon has been extensively researched in relation to the regular dietary consumption of folic acid and the prevention of neural tube defects during embryogenesis in humans. Folic acid or folate is a water-soluble vitamin of the B complex which plays a critical role in cell division and the formation of new cells - two main processes that are vital during prenatal development and the maturation of undifferentiated cells. Folate is important to numerous bodily functions, ranging from nucleotide synthesis to the reduction of elevated plasma homocysteine levels. It naturally occurs in leafy vegetables such as spinach and lettuces, but as more research is conducted regarding the nutrient's benefits on health and wellness, its discovery has influenced food scientists and processing experts to add folate to a variety nutraceutical supplements and fortified foods. Furthermore, numerous reports have suggested that nutritional deficiency in general, and folate deficiency in particular, can cause adverse birth outcomes such as neural tube defects (Bendich & Butterworth, 1996).

A neural tube is an early embryonic precursor of the central nervous system, comprising of the brain and spinal cord. After a short period prior to its complete formation, the neural tube is open both cranially and caudally (Dehner & Stocker, 2001). Improper closure during the fourth week of pregnancy can result in neural tube defects such as spina bifida, anencephaly, and encephalocele (Dehner & Stocker, 2001). It is during this crucial point in embryogenesis where folate acts as a substrate for enzymes involved in DNA and RNA biosynthesis – biological processes that are essential to these nascent tissues. Overall, the underlying purpose of this report is to distinguish the relationship between folate consumption and neural tube formation and to discuss many of today's accepted mechanisms describing the role of folate in the prevention of neural tube defects.

Folates are involved in a large number of biochemical processes, such as amino acid metabolism, purine and pyrimidine synthesis, and methylation of a large number of nucleic acids, deoxyribonucleic acid, proteins, and lipids (Alberto *et al*., 2007). Folate is particularly important in the homocysteine/methionine cycle. Over the past decade, there has been growing evidence that even a moderately elevated increase in homocysteine can increase the risk of prenatal abnormalities, particularly neural tube defects (Herrmann, 2001). Homocysteine is a homologue of the amino acid cysteine which is used by the body to help manufacture proteins and carry out cellular metabolism. In order to fully comprehend the link between folate and early prenatal development, it is necessary to examine the biochemical roles of folate in homocysteine metabolism. When homocysteine accumulates in cells, it is removed by remethylation into the amino acid methionine or by a process known as *trans-sulfuration* into cysteine (Alberto *et al*., 2007). In the latter mechanism, homocysteine is condensed with the amino acid serine in a reaction catalyzed by the enzyme cystathionine-β-synthase to form cystathionine, which in turn, is reduced to cysteine and α-ketobutyrate by the enzyme cystathionine lyase. Both of these enzymes depend on an active form of vitamin B_6 (Alberto *et al*., 2007).

During the remethylation into methionine, a methyl group is transferred from 5-methyltetrahydrofolate to homocysteine via the enzyme methionine synthase. For this reaction to take place, vitamin B_{12} must be present since it acts as an intermediate molecule that carries the methyl group. The resulting methionine will then be used either in the formation of peptides during translation or transformed by the enzyme methionine adenosyltransferase into *S*-adenosylmethionine, which transfers an adenosyl group from ATP to methionine. *S*-adenosylmethionine plays a significant role in cellular functions because it serves as a universal methyl donor in a large number of methylation reactions. This cycle is highly dependent on the folate cycle; in fact, this cycle could not turn without the folate cycle. The methyl-donating 5-methyltetrahydrofolate is converted from 5,10-methylenetetrahydrofolate via the enzyme 5,10-methylenetetrahydrofolate reductase. After homocysteine has been remethylated to methionine, tetrahydrofolate lacking a methyl group will be converted back into 5,10-methylenetetrahydrofolate (Alberto *et al*., 2007). Therefore, methylenetetrahydrofolate reductase has a central

function in the folate cycle, especially since it directs dietary folate towards the remethylation of homocysteine for DNA and RNA synthesis (Fowler, 2001). In addition, methylenetetrahydrofolate reductase can also be used in several one-carbon transfer reactions during the synthesis of thymine, as well as during the synthesis of purines (Alberto *et al*., 2007).

Since all these metabolic processes are interwoven, any imparity would lead to dramatic biochemical variations in terms of homocysteine levels circulating within the growing fetus. In a study conducted by Blom *et* al. (1995), it was discovered that a single nucleotide polymorphism exists for the enzyme methylenetetrahydrofolate reductase gene, characterized by an alanine-to-valine substitution at position 222, resulting in a 50% reduction in enzyme activity. Consequently, the polymorphism has been associated with elevated levels of homocysteine, particularly in patients with insufficient folate intake. When the frequency of this mutation was further analyzed by Blom *et al*. (1997), it was observed that this gene was more prevalent in mothers who gave birth to infants with neural tube defects. In fact, it was shown that if the offspring also inherited this gene, that is, both mother and her child are homozygous for the mutation, the risk of spina bifida increased 6 to 7-fold (Blom *et al*., 1997). However, the effect of this gene can be reversed by additional folic acid intake (Boers *et al*., 1998).

Recall that if the reductase enzyme is unavailable, tetrahydrofolate can no longer attain another methyl group in order convert homocysteine into methionine. Methionine is an essential amino acid that cannot be synthesized by the body. In fact, nearly all mRNA encode methionine as their initial amino acid to begin translation. Besides protein synthesis, methionine is involved in the formation of *S*-adenosylmethionine, which is a substrate for many transmethylation reactions, such as DNA methylation (Blom *et al*., 2001). DNA methylation can change the properties of chromatin, such as its structure and activity, and is associated with a number of key processes including the repression of gene expression - a process that allows cells to become determined and possess specialized roles in varying tissues. It is during the early stages of embryogenesis where DNA methylation is undergoing its most rapid changes (Boubelik *et al*., 1987). In an experiment conducted by Choi *et al*. (2000), it was discovered that in homozygous methylenetetrahydrofolate reductase patients, the resulting deficit in 5-methylenetetrahydrofolate was associated with a lack of DNA methylation in peripheral blood mononuclear cells. Therefore, a shortage in the supply of methyl groups or any small delays as a result of dietary folate deficiencies or folate-associated enzyme deficiencies could result in impaired methylation of DNA, which in turn, could lead to improper gene expression of undifferentiated cells encoding for the receptors associated in cell adhesion during neural tube closure.

During neural tube formation, cells destined to becoming the neural tube undergo a primary and secondary neurulation phase. In primary neurulation, the cells of the neural plate invaginate from a flat surface into a cylindrical neural tube. Secondary neurulation refers to the sequential process where the cells of the neural plate form a massive neural cord that migrates inside the embryo and hollows to form the tube (Blom *et al*., 2001). In an experiment performed by Ames *et al*. (1997), uracil levels in DNA in folate-deficient and folate-sufficient groups were accurately determined to study the role of uracil misincorporation in DNA damage induced by folate deficiency. It was discovered that folate deficiency caused massive incorporation of uracil, as opposed to thymine, into human DNA (4 million per cell) and induced chromosome breaks. They proposed that the likely mechanism is due to the deficient methylation of deoxy*uridine* monophosphate to deoxy*thymidine* monophosphate and subsequent incorporation of uracil into DNA by polymerase. Therefore, if folate is deficient in a person's diet, namely one who is pregnant, there is likely to be a decrease in the cellular synthesis of 5,10-methylenetetrahydrofolate, as well as reduced methylenetetrahydrofolate reductase activity, which in turn, would lead to an increase in deoxy*uridine* monophosphate, and thus to an excessive incorporation of uracil into DNA, with the subsequent repair mechanisms increasing the risk of chromosome breakage. Without adequate amounts of thymine, DNA cannot be produced in cells that make up the neural tube, which inevitably would lead to a defective end-product. Therefore, although folate does not have a direct effect on DNA, its presence indirectly allows processes which lead to DNA synthesis to take place, which is why adequate folate is crucial for neural development and function.

It is quite evident that folate is an important factor in the biochemical processes that lead to neural tube formation; however, if supplementation is not taken at the right time, pregnant women cannot reap its full benefits. The neural tube closure generally occurs on days 22-28 after ovulation (Economides & Kadir, 2002). Since more than 40% of pregnancies are unplanned most of these women are unaware of being pregnant up until the first or second month in their pregnancy (Economides & Kadir, 2002). In a study performed by Schorah *et al*. (1976), vitamin levels

in blood of first trimester women who had a baby with neural tube defects were measured. They discovered that *red blood cell* folate were significantly lower compared to mothers without neural tube defected infants, even though their *serum* folate was normal. Recall that red blood cells have a life span of 120 days; this suggests that the red blood cells of these women originated well before conception, whereas serum folate reflects recent dietary intake. Therefore, even if adequate amounts of folate are ingested during the first few weeks into pregnancy, what matters more is if adequate amounts of the vitamin are ingested prior to pregnancy than during pregnancy (Schorah *et al*., 1976). This implies that periconception ingestion of folate can reduce the number of pregnancies affected by neural tube defects (Berry *et al*., 1988). Currently, 400 µg of folic acid is recommended for women in the general population who are trying to conceive (Economides & Kadir, 2002).

Studying folate metabolism helps scientists and medical physicians understand why neural tube defects arise in certain pregnancies. These studies help women who are trying to conceive understand the risks of exercising certain diets before and during their pregnancy. Given the available evidence, women who take these precautions can feel relaxed that they have done all they can to ensure that their developing child has a lower chance of being inflicted with a folate-deficient cause of a neural tube defect. In fact, knowing the culprit behind these defects potentially avoids or minimizes the number of pregnancy terminations in the second trimester because of these anomalies that prevent the child from developing normally. On the basis of these findings, the government can also take the immediate precautions of determining the proper recommended amounts for people of the general public and persuade food makers to fortify foods with folic acid as a justifiable means of preventing neural tube defects from occuring in women with unplanned pregnancies who, at the same time, are uneducated about this phenomenon.

Future studies should focus on identifying polymorphisms or mutations in genes involved in the synthesis of thymidylate, purines, regulatory proteins, or substrates involved in folate and homocysteine metabolism, such as serine hydroxymethyltransferase and methylenetetrahydrofolate dehydrogenase, rather than simply focusing on 5,10-methylenetetrahydrofolate reductase as the main cause. These studies should investigate mutations within the coding regions of the genes, quantification of mRNA levels, examination of untranslated regions of the gene, and test promoter functions (Blom *et al*., 2001). Moreover, most of the current studies only verify the mechanism of an impaired homocysteine metabolism in relation to a defective neural tube closure, but still give no insight in the mechanisms that are affected during neurulation. Henceforth, in order to be able to stipulate a vivid link between a disruption in homocysteine metabolism and neural tube defects, studies should focus on the involvement of homocysteine in microfilament synthesis or processes leading to DNA methylation. Lastly, research should also focus on non-folate alternatives in preventing neural tube defects, since only 30% of neural tube defected pregnancies are due to dietary folate deficiencies (Blom *et al*., 2001). If these factors are not taken into full consideration and detailed accurately, major pieces of the puzzle still await discovery and explanation. Overall, dietary folate should be accepted as an important means of helping to keep a developing infant and his/her mother healthy.

References

Alberto J.M., Daval J.L., Forges T., Guéant J.L., Guéant-Rodriguez R.M., Monnier-Barbarino P. (2007). Impact of folate and homocysteine metabolism on human reproductive health. *Human Reproductive Update*, 13(3): 225-238.

Ames B.N., Blount B.C., Everson R.B., Hiatt R.A., Mack M.W., MacGregor J.T., & Wickramasinghe S.N. (1997). Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *The Proceedings of the National Academy of Sciences USA*, 94: 3290-3295.

Bendich A., & Butterworth C.E. (1996). Folic Acid and the Prevention of Birth Defects. *Annual Reviews of Nutrition*, 16: 73-79.

Berry R.J., Cordero J.F., Erickson J.D., & Mulinare J. (1988). Periconceptional use of multivitamins and the occurrence of neural tube defects. *The Journal of the American Medical Association*, 260: 3141-3145.

Blom H.J., Boers G.J.H., Den Heijer M., Frosst P., Goyette P., Matthews R.G., Milos R., Sheppard C.A., & Van Den Heuve L.P. (1995). A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Natural Genetics*, 10: 111-113.

Blom H.J., Esker T.K., Graaf-Hess A., Mariman E.C.M., Smeitinik J.A.M., Steegers-Theunissen R.P.M., Thomas C.M.G., & Can Der Put N.M.J. (1997). Altered folate and vitamin B12 metabolism in families with spina bifida offspring. *QJM: An International Journal of Medicine*, 90: 505-510.

Blom H.J., Trijbels F.J.M., Van Der Put N.M.J., & Van Straaten H.W.M. (2001). Folate, Homocysteine and Neural Tube Defects: An Overview. *Experimental Biology and Medicine*, 226(4): 243-270.

Boers G.H.J., Eskes T.K., Nelen W.L., Steegers E.A., & Thomas C.M. (1998). Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. *Jounral of Nutrition*, 128: 1336-1341.

Boubelik M., Lehnert S., & Monk M. (1987). Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development*, 99: 371-382.

Choi S.W., Mason J.B., Selhub J., & Stern L.L. (2000). Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. *Cancer epidemiology, biomarkers & prevention*, 9: 849-853.

Dehner L.P., & Stocker T.J. (2001). Pediatric Pathology (1st ed.). *Lippincott Williams & Wilkins*.

Economides D.L., & Kadir R.A. (2002). Neural Tube Defects & Periconceptional Folic Acid. *Canadian Medical Association Journal*, 167(3): 255-256.

Fowler B. (2001). The folate cycle and disease in humans. *Kidney International*, 59: 221-229.

Herrmann W. (2001). The importance of hyperhomocysteinemia as a risk factor for diseases: an overview. *Clinical Chemistry and Laboratory Medicine*, 39: 666-74.

Roche H.M. (2006). Nutrigenomics - new approaches for human nutrition research. *Journal of the Science of Food and Agriculture*, 86(8): 1156-1163.

Schorah C.J., Sheppard S., & Smithells R.W. (1976). Vitamin deficiencies and neural tube defects. *Archives of Disease in Childhood*, 51: 944-950.