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Neural tube defects (NTD) are the most common types of birth defects. Research shows that folic acid taken periconceptionally greatly reduces the risk of having a NTD affected child. This paper will explain the role that folate plays in the metabolism, specifically in synthesizing methionine. It will bring evidence to show that methionine is crucial for normal neural tube development. In addition, it will explore the genetic factor involved in folate metabolism and possible folate deficiency.

The central nervous system in an embryo begins as a flat region, which then rolls into a tube known as the neural tube. The development of the neural tube is completed 28 days after the baby is conceived. When the neural tube fails to close completely, it is known as a neural tube defect. The nerves that are exposed to the environment due to the failure of the tube to close may become damaged causing the affected baby to have a disability, specifically some measure of paralysis. Neural tube defects are one of the most common birth defects, and affects 1.3- 2.0 babies in every 1,000 live births in the United States. There are two main types of NTDs, depending on whether the cranial or caudal end of the neural tube fails to fuse properly. The foremost cranial defect is known as anaencephaly which is usually fatal. The main caudal defect is spina bifida (Pitkin, 2007).

The exact causes of NTDs are not clear. It is believed that a combination of environmental factors, genetics, and nutrition contribute to the development of a NTD. However, as early as the 1970 scientists have suspected that folic acid taken by the pregnant mother helps prevent NTDs. In the United Kingdom, R. W. Smithells noticed that a disproportionate amount of babies affected with NTD were born to mothers of a lower socioeconomic class. He hypothesized that the birth defects may have been caused by the mothers' poor nutrition. He conducted tests and in 1976 reported that women who had a child with an NTD had much lower red cell folate and vitamin C levels than mothers who had an unaffected child (Smithells, Sheppard, & Schorah, 1976). He then conducted a placebo-controlled study in which he gathered women who had already given birth to a NTD affected child. He gave one group of women a multivitamin and an iron tablet containing 360 ug of folic acid. The group of women who were given the vitamin pill only had a .5% occurrence of NTD births, while the control group had a 4% NTD birth rate incidence (Smithells et al, 1981). Smithells results were published, however his experiment was criticized because his subjects had not been randomized.

K. M. Laurence in Whales also performed experiments with folic acid based on Smithells' works. He performed a double blind randomized controlled trial of folate treatment prior to conception. Among the group that received the folate, there were no NTDs in the group's 44 births. However, in the control group there was 6 NTDs in 67 births. Laurence's work was not considered statistically significant since it was performed on such a small population (Mills et al. 1996).

Smithells' findings were eventually proved definitively by the Medical Research Council in 1991. The MRC conducted a double-blind randomized study on women who had a previous NTD pregnancy.

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Women received 4 mg of folic acid, a multivitamin, both, or iron and calcium. There was a 74% reduction of NTD births in the group taking folic acid, but no effect in the groups that did not receive folic acid. Another study, conducted just one year later also helped to validate Smithell's works. Czeizel and Dudas in Hungary enrolled women who had never had an affected pregnancy. They were randomized and given a multivitamin, some with .8 mg folic acid, and some without. The group of 2394 women who were given folic acid had no affected offspring, and the group of 2310 women who did not receive folic acid had 6 babies with NTD (Mills et al. 1996).

Due to the solid evidence of the protective role of folic acid, public health officials recommend women of childbearing age to take 400 ug of folic acid at least 4 weeks before conception, and through the first trimester of pregnancy. Folic acid is the oxidized and most active form of folate. They may do this by taking a folic acid supplement. They are also encouraged to eat folic acid enriched grain products and large amounts of folate rich foods such as green leafy vegetables, or liver. The United States, as well as other countries, have implemented a mandatory folic acid food fortification and have seen about a 50% reduction in the occurrence of NTDs (Lamers, Prinz-Langenohl, Bramswig, Pietrzik, 2006).

Despite the awareness of the role that folic acid plays in preventing NTDs, the exact mechanism used to achieve this is not so clear and is still being studied. However, based on various experiments scientists have come to a basic understanding as to how a folate deficiency would contribute to an NTD development.

Folate is a term to describe a water- soluble B-complex vitamin and it serves many important roles in the body. It is vital for cell division and homeostasis due to the essential role of folate coenzymes in nucleic acid synthesis, methionine regeneration, and in the shuttling, oxidation and reduction of one carbon units required for normal metabolism and regulation. There is an increase in folate requirement in pregnant women to support the rapid growth of the embryo and uteroplacental organs. There has been much focus on the role folate plays in methionine regeneration in regard to studies of NTDs. In this process folate is reduced in the body to dihydrofolate and then to tetrahydrofolate. Tetrahydrofolate acts as an acceptor molecule that accepts and donates one carbon units in metabolic pathways. A one carbon unit is transferred from serine to THF by serine hydroxymethyltransferase to form 5,10 methylene tetrahydrofolate. This compound is then reduced by methylene tetrahydrofolate reductase (MTHFR) to form 5 methyl tetrahydrofolate. The 5 methyl tetrahydrofolate is acted upon by methionine synthase together with a B-12 cofactor which facilitates the removal of the N-5 methyl group from the 5 methyl tetrahydrofolate and deposits it onto homocysteine. The homocysteine with the extra methyl group is known as methionine. A mother who is lacking in folate would not be able to carry out this metabolic pathway, since 5 methyl tetrahydrofolate is the only compound capable of this one carbon transfer (Bailey, & Gregory, 1999). It would therefore seem plausible to say that mothers lacking in folate, and therefore at risk for an NTD baby, would have elevated homocysteine levels and low methionine levels. Subsequent studies have indeed supported this theory.

Steegers- Theunissen et al. (1995) was one of the first to report elevated amniotic fluid homocysteine in women who were carrying an NTD fetus. Since then there have been many reports that

plasma or amniotic fluid homocysteine is higher in NTD infants and their mothers than non-NTD infants and their mothers. One such study was conducted by Mills in which blood samples were collected during pregnancy from women carrying affected fetuses, and random women carrying healthy fetuses. The homocysteine levels in the blood were measured, and the mothers if NTD fetuses had significantly higher levels. These studies pointed to the relative inability of mothers with NTD babies to metabolize homocysteine (Mills et al. 1996).

Since it has been established that NTD mothers have higher levels of homocysteine, research turned to try to establish if too much homocysteine is what actually inhibits the neural tube closure. In order to test this hypothesis Greene, Dunlevy and Copps (2003) cultured mouse embryos in the presence of homocysteine thiolactone during the periods of cranial neural tube closure. While the homocysteine thiolactone did cause growth retardation and other negative effects, it did not increase the incidence of neural tube defects. Another study conducted by Afman, Blom, Van Der Put, and Van Straaten (2003) and his colleagues administered homocysteine to chick embryos in ova. This resulted in several malformations, but did not increase the number of NTDs. These results suggest that too much homocysteine is unlikely to be the direct cause of NTD.

Since the overabundance of homocysteine in a mother of an NTD child does not seem to cause the NTD, perhaps the lack of methionine that should have been made from the homocysteine plays a role in the development of an NTD. Methionine is an essential amino acid that cannot be obtained sufficiently through dietary intake and therefore must be synthesized by the body. Methionine formed from homocysteine is converted to S- adenosylmethionine, which is a methyl donor for many reactions including DNA methylation (Friso et al. 2002). This theory was tested in a few different ways. Coelho, Weber, Klein, Daniels, and Thomas (1989) conducted a study in which he grew rat embryos in cow serum. Cow serum has a much lower level of methionine than rat serum. They supplemented some cow serum with methionine, and did not supplement others. There was a significant decrease in the occurrence of NTD in the embryos that were grown in the serum that was supplemented with methionine. Essein and Wannberg (1993) conducted a study involving pregnant mice with an Axd (axial defect) mutation which is known to cause NTDs. They injected these pregnant mice with methionine on days 8 and 9 of the pregnancy. At a dose of 180 mg/ kg body weight the methionine produced a 47% reduction of NTDs. They also conducted studies with mice with the Axd mutation in which they supplemented the mice with folinic acid, but it had no effect on the incidence of NTDs. This seems to suggest that folate, or lack of it, is an indirect cause of NTD, and methionine is the needed product of the folate for normal neural tube development. Perhaps the Axd mutation codes for a mutation in an enzyme that is crucial to make methionine, or a vital product of methionine, but is not involved with folate at all.

Shaw, Velie, Schaffer (1997) conducted a study concerning the effect of methionine on humans. His study involved 424 mothers of NTD children, and 440 mothers of unaffected children. Each mother was interviewed in which they answered a 100 item food frequency questionnaire. The data was then established into quartiles of average daily maternal dietary intake of methionine in the 3 months before conception. There was a 30- 40 % reduction in NTD affected pregnancies among women whose average daily intake of methionine was above the lowest quartile. These observations were unrelated to the

maternal level of folate intake, which supports the theory that methionine, or what methioine is converted to, is what is actually crucial for normal neural tube development.

Assuming that methionine is vital for normal embryonic development, and the synthesis of methionine is formed by homocysteine and 5 methyl tetrahydrofolate interaction, then it is clear how a folate deficiency would result in a NTD. However, many studies since Smithells have reported that NTD mothers are not necessarily folate deficient. One such study was conducted in Molloy and Kirke, Hillary, Weir, and Scott (1985). They studied the serum samples taken during pregnancy from 32 mothers with pregnancies affected by NTD and 395 randomly selected unaffected pregnant women. The serum folate levels and vitamin B-12 levels (vitamin B-12 is the coenzyme that facilitates the removal of a methyl group from the 5 methyl tetrahydrofolate onto the homocysteine) were measured and analyzed. To analyze the data the information was sub- classified into folate deficient, possible deficient, and sufficient ranges. The ranges were 0-2 ng/ml, 2.01- 2.7 ng/ml, and 2.71-20 ng/ml respectively. Surprisingly, only 21.9% of the NTD group was deficient in either folate or vitamin B-12 while 22.8% of the control group samples were. 43.8% of the NTD group, and 35.7% of the control group showed sufficient serum concentrations of both folate and vitamin B-12. These results show that a significant percent of women who had folate sufficient levels had a pregnancy affected by NTD. This seems to indicate that folate deficiency may not be the sole or main cause of neural tube defects. There may be a more subtle problem among women who gave birth to NTD babies than simply not consuming enough folate as part of their diet. These results do not contradict Smithells' findings, since he reported that NTD mothers have lower red cell folate levels. This study was only able to measure the serum folate levels, and this difference seems to be significant.

Red cell folate levels and serum folate levels were further investigated in other studies. Once such study was conducted by Yates et al. (1986) measured vitamin levels in twenty women less than 35 years of age who had two or more NTD pregnancies. Each case was compared to a control female who was matched for age, obstetric history, and social class. The red cell folate levels were measured and showed a linear relationship with the number of NTD pregnancies. However, there was no significant difference between the subjects and controls in relation to serum folate levels. The diets of the study and control women were ascertained through a questionnaire. There was no statistically significant difference between the folate dietary intakes of the two groups of women. However, regression analysis showed a difference between the two groups in regard to the relationship of red cell folate to dietary folate. This study provides additional evidence that low red cell folate is linked to NTD, but also demonstrates that low red cell folate is not necessarily due to a folate deficient diet. The difference between the red cell folate levels among women with similar folate intake supported the increasingly popular idea that NTD may be linked to a disorder in folate metabolism, and not exclusively to a folate deficient diet.

More recently, in 1992 Mills et al. conducted a study which measured the maternal serum folate of 89 NTD pregnancies and 178 control pregnancies. This study also demonstrated no relationship between maternal serum folate during pregnancy and the risk of NTDs (Mills et al. 1996).

There is also a strong hereditary link for neural tube defects. The chance of having a NTD child is about 0.15 percent. However, once a woman has a child with a NTD the chance of having a second child with NTD is increased to about 2-5%. Furthermore, if the women herself has a NTD the chances of having an affected baby increases. This supports the idea that there may be a genetic mutation that predisposes someone for NTD among those who are affected by it (Genetics and Neural Tube Defects, 2005).

Due to the evidence provided by studies such as the ones mentioned above, there has been much speculation that there is possibly a genetic defect that affects the metabolism of folate. To prove this hypothesis researchers have identified several common polymorphisms of genes that code for folate metabolizing enzymes, including the 677C / 677T and the 1298A/ 1298C alleles of 5, 10 methylenetetrahydrofolate reductase (MTHFR). These polymorphisms are common, and their frequency varies by race and ethnicity. A recent study by Yang et al. (2008) tried to determine the role that these different alleles play in the metabolism of folate and homocysteine levels.

Data for this study was taken from the third National Health and Nutrition Examination Survey (NHANES III). The NHANES III endeavored to obtain a nationally representative sample of the civilian United States population. Each survey participant was given an interview, a physical examination, and gave a sample of their blood. The researchers genotyped selected polymorphisms of folate metabolizing enzymes in DNA samples (obtained from the blood specimen) for 7159 individuals. They also measured the serum folate levels and homocysteine levels in each blood sample. Based on the interviews of the participants their average daily intake of folate was determined. The effects of MTHFR 677C/677T genotype and the MTHFR 1298A/1298C genotype on the inverse relationship between folate intake and serum homocysteine concentrations were then examined.

The researchers observed significant differences in the serum folate and homocysteine concentrations for individuals with the MTHFR 677 TT genotype. The adjusted geometric mean of serum folate concentration was 24.83 % lower if they had the TT genotype that if they had the CC genotype. The adjusted geometric mean of serum total homocysteine concentration was 29.06% higher if they had the TT genotype than if they had the CC genotype. The other polymorphism, 1298A/1298C, was not significantly associated with serum folate or homocysteine concentrations.

Folic acid consumption also played an important part in determining serum folate levels. Those with the 677 TT genotype that took 400 ug of folic acid per day had higher serum folate concentrations than those with the MTHFR 677 CC who did not take folic acid supplements. As the folic acid consumption increased, the difference between the levels of serum folate of the TT and CC genotypes decreased significantly. The difference actually became non significant among the group who took 400ug or more of folic acid per day. However, homocysteine levels among the MTHFR 677TT only decreased by 11-14% with the supplemental folic acid.

These results seem to indicate that having 677 TT alleles on the gene that codes for MTHFR results in some sort of defect on the enzyme which causes lower folate levels than in someone who has

the same folate dietary intake but has the 677 CC alleles. However, consuming a lot of folic acid, specifically 400 ug per day, can overcome the problem and bring the serum folate to sufficient levels.

The exact explanation for what the polymorphism codes for, and how the folate is able to overcome the defect can be explained. The 677T polymorphism occurs in exon 4 of the genetic code and results in a valine substitution of alanine at codon 222. This valine lies on the binding site for the MTHFR enzyme's cofactor flavin adenine dinucleotide (FAD). This affects the binding of the FAD to the enzyme, making the binding site exposed instead of imbedded in a barrel-like structure. The exposure results in a weakened, thermolabile MTHFR/FAD complex and the MTHFR 677TT enzyme has been shown to dissociate with the FAD cofactor more readily than the MTHFR 677CC enzyme. This results in decreased enzymatic activity. The MTHFR enzymes with the 677TT genotype have a 30% in vitro MTHFR enzyme activity as compared to the MTHFR 677CC enzymes. However, an abundance of 5-methyltetrahydrofolate substrates have been shown to strengthen the complex and protect the MTHFR from losing the FAD cofactor. This explains how consuming an abundance of folic acid overcomes a natural genetic deficiency (Robien, & Ulrich, 2003).

The results of the study by Yang et al. explains the findings in the studies which show that people of similar folate intake may have different red cell folate levels. However, in the those studies, the women who had given birth to NTD children did have sufficient serum folate levels, while in the study by Yang the MTHFR reduced activity was specifically reflected in low serum levels. It is possible that this can be explained simply by the different states of the subjects of the studies. The studies that showed similar serum levels were conducted on pregnant women. It has already been established that pregnant women are in high need of folate. The plasma is the provider of folate during pregnancy and therefore the body does al it can to ensure sufficient serum folate. When the maternal serum folate reaches deficient levels, folate is taken from the red cells that serve as folate storage tissue (Lamers, Prinz-Langenhol, Bramswig, & Pietrzik, 2006). That would explain the consistent findings of low red cell folate levels, but sufficient serum folate levels of the NTD mothers. However, the study of polymorphisms was conducted on a representational population of United States residents, and therefore presumably only has a small percent of pregnant women. Since folate is not as desperately needed among non-pregnant people, the body has no urgent need to bring up the serum folate levels, and therefore they remain deficient.

If the 677TT MTHFR polymorphism is actually what leads to low serum folate and high total homocysteine levels, perhaps it would be more efficient to simply supplement the body with what the MTHFR should be synthesizing rather than with folic acid which stabilizes the enzyme. This speculation was proven true in a study by Lamers et al. (2006). The study involved 144 women aged 19-33 who were not pregnant or lactating. The study was a 24 week double-blind placebo-controlled trial. The women received either 400ug of folic acid, 416 ug [6S] 5 methyltetrahydrofolate, 208 ug [6S] 5 methyltetrahydrofolate, or a placebo daily. Blood samples were collected every 4 weeks of the study. At baseline, the 4 groups did not differ significantly with respect to red cell and serum folate concentration. No change in dietary intake was observed throughout the study period. Although all 3 groups receiving supplements showed significant red cell and serum folate increases, the increase in red cell folate and

serum folate was significantly greater in the group receiving 416 ug [6S] 5 methyltetrahydrofolate than the other 2 groups. The group that received 400 ug of folic acid had the second highest increse.

This study proves that supplemental [6S] 5 methyltetrahydrofolate at approximately the same dosage as supplemental folic acid is more efficient than folic acid at raising folate levels. This supports the idea that the lag in the folate metabolism present in some people occurs at the MTHFR enzyme which is supposed to synthesize 5 methyltetrahydrofolate. Therefore providing the body with the product of the enzyme overcomes the defect more efficiently than trying to stabilize the enzyme with additional folic acid.

The role that folic acid plays in the prevention of the occurrence of a NTD is well documented and proven. Folic acid aids in the deposition of a methyl group on homocysteine, thereby creating methionine. Methionine is then converted to other substances, such as S- adenosylmethionine, which is essential for normal embryonic development. Pregnant women who are folate deficient may therefore not have sufficient methionine levels to support a growing embryo. This could lead to birth defects, such as the failure of the neural tube to close properly. Pregnant women may be folate deficient due to a poor diet. However, even women who do have a folate sufficient diet may have low red cell folate serum levels if they have the 677TT MTHFR polymorphism. This genetic defect can be overcome by consuming an abundance of folic acid, beyond what is normally considered sufficient. Today there is much effort expended on educating the public about the beneficial role of folic acid, and encouraging women of child bearing age to take folic acid supplements.

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