Mediation of Folate Transport across the Placenta

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Abstract

Folate (B9) is an essential molecule for the development and maturation of cells. During gestation, it is extremely important to the growth and development of the nervous system. Systemic deficiencies of B9 during pregnancy cause neural tube defects (NTD) ranging from spina bifida to anencephaly. However, over supplementation of folate has been linked with the progression of cancer and therefore is not a viable means for some patients. If inhibition of the transporter is caused by autoantibodies, supplementation with omega-3 may reduce inflammation and thereby increase folate transport without supplementation of folate. An epidemiological study should be performed to test this hypothesis. A program was created that joins the National Birth Defect Prevention Study (NBDPS) database to the USDA database to analyze the correlation between individual intake of omega-3s and omega-6 and birth outcome. The program exclusively uses NBDPS data and allows better access to the outdated database.

Specific Aims

Folate is essential for cell function as a methyl carrier. During fetal development, it aids in neural development and neural tube closure. B9 deficiencies during pregnancy cause neural tube defects (NTD), where the neural tube fails to close, ranging from spina bifida to anencephaly. Supplementation with folate, in addition to fortified food, is recommended. However, over supplementation of folate has been linked with increased cancer progression and therefore is not a viable means for some patients. NTDs have been linked to folate transporter autoantibodies. Reducing this immune response may result in improved birth outcome. Supplementation with omega-3, potent anti-inflammatory, may decrease T cell and B cell upregulation. This in turn would increase folate transport.
To test DHA and EPA supplementation on birth outcome, a program was created that compares full birth outcomes and diets from 15000 participants from the NBDPS. To obtain fatty acid data, the foods were run through the USDA database for DHA, EPA, α-LNA, linoleic acid, AA.

This experiment may have large benefits for the pregnant community. For individuals who are currently cancerous, or at high risk, supplementation with folate is not a viable option. By finding an alternative, these groups are put at a lower risk. In addition, conditions like CFD may be treated via this method.

Introduction

This paper will discuss the role of omega-3 and omega-6 in autoantibody inhibition as pertaining to folate transfer, with the goal of exploring an alternative to folate supplementation. The discussion begins with basic knowledge considering folate including the folate cycle, transporters, and homeostasis. Next, it moves into consequences of deficiency (Folate and NTDs) and over supplementation (Folate and Cancer). A brief introduction to antibody production is given, and then the role of antibodies in folate transporter inhibition is discussed. Finally, mediation of autoantibodies by omega-3, omega-6, their derivatives, and NSAIDs are explained. Proposed methodology to test the hypothesis is mentioned with concluding remarks.

Folate Cycle

![Figure 1: Folic Acid Cycle](image)

Folate and folic acid (dietary forms) enter the cycle as THF. THF turns into MTHF via MTHFR. Using the MTR or methionine synthase (MSR) MTHF donates a methyl group to homocysteine, making methionine. This activates SAM, turning methionine back into homocysteine.
Folate, vitamin B9, is an essential nutrient for the growth and differentiation of many cells, especially neural tissue. Although folate and folic acid are the dietary forms, the biologically active form of B9 is tetrahydrofolate (THF). Dihydrofolate reductase converts B9 into THF inside the liver. Inside the folate cycle, THF quickly converts into 5,10-methylenetetrahydrofolate. This is reduced to 5-methyltetrahydrofolate (MTHF) via the action of methylenetetrahydrofolate reductase (MTHFR) and NADPH. MTHF, via 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR) converts homocysteine into methionine, oxidizing MTHF back THF\textsuperscript{8,9}.

The reduction of homocysteine is incredibly important to biological homeostasis. Increased levels of homocysteine are associated with heart problems like megaloblastic anemia, atherosclerosis, heart attack, stroke, and venous thrombosis\textsuperscript{10}. Hyperhomocysteinemia patients also see neurological problems like cognitive impairment, dementia, depression, and in rare cases peripheral neuropathy\textsuperscript{10}. One of the end goals of homocysteine reduction is the activation of SAMe. SAMe activates S-Adenosyl methionine (SAM) a potent methyl carrier. SAM is required for biosynthesis, protein modification, DNA methylation, tRNA modification, lipid metabolism, methyl donation, and cell activation\textsuperscript{4,8,9}. SAM is the end goal of the folate cycle and is responsible for many cells’ differentiation and maturation processes via DNA and RNA methylation\textsuperscript{9}.

### Folate Transport

Three human transports are responsible for folate: the reduced folate carrier, the folate receptor, and the proton coupled folate transporter. Reduced folate carrier (RFC) uses a secondary active antiporter to transport folate into cells from circulation. Folate receptor (FR) is a receptor-mediated endocytotic transporter. The proton-coupled folate transporter (PCFT) is pH and substrate dependent. Each of these transporters have varying importance in different locations of the body\textsuperscript{11}.

**The Reduced Folate Carrier**

The RFC is the primary folate transporter. The constitutively expressed phosphate-folate antiporter is composed of 591 amino acids and 12 transmembrane domains. The domains are divided in half by a large intracellular loop. Both the N- and C- termini face the cytoplasm. The expression of folate transporters is sensitive to dietary folate, as low-folate diets increase RFC transporters in the small intestine\textsuperscript{11}.

RFC is optimal at a neutral pH of 7.4 and has a relatively low binding affinity compared to the other transporters. At low pH, this decreases more. Folic acid
binds at 1/100 the rate of methotrexate (MTX), a folate antagonist, and PT523, a powerful antifolate, binds at a specificity > 10x MTX. Therefore, RFC is preferential to antifolates at low pHs\textsuperscript{11}.

RFC is required for embryonic development. Inactivation of both alleles is lethal to mice fetuses. Supplementation with folate allows live birth of the mice, only to have them die shortly after\textsuperscript{11}.

**High-Affinity Folate Receptors**

Three isoforms of the folate receptor (FR) exist: FR\textalpha, FR\textbeta, and FR\textdelta. FR\textalpha is expressed on epithelial tissues while FR\textbeta is expressed in placenta, hematopoietic tissues and activated macrophages. FR\textdelta is expressed on T regulatory cells (both natural and TGF\textbeta-induced)\textsuperscript{11}.

FR\textalpha and FR\textbeta transport folate via a receptor-mediated endocytosis. In this process, folate binds to FRs at the cell membrane, which then envelopes the ligand-receptor complex. The envelope buds off to form a vesicle. The folate is acidified, released from the receptor, and then exits the vesicle to enter the cytoplasm\textsuperscript{11}.

Absence of FR\textalpha alleles is lethal at the time of neural tube closure. Partial folate supplementation, before and during gestation, results in fetuses with a variety of developmental defects. With total gestation supplementation, normal offspring are delivered that do not require further folate supplementation. Therefore, FR\textalpha's essential functions are embryonic and fetal development across specialized epithelial tissue\textsuperscript{11}.

**Figure 2. Folate Transporters\textsuperscript{7}**.

On the far left is the RFC transporter that imports folate while exporting organic phosphates. The middle shows PCFT, which uses the hydrogen ion gradient to import folate. On the far right is the folate receptor-mediated endocytosis.
The Proton-Coupled Folate Transporter

Proton-coupled folate transporter (PCFT) is a voltage-gated cotransporter composed of 459 amino acids and 12 transmembrane domains. PCFT is expressed in the kidney, liver, placenta, and spleen, and, to a lesser extent, the brain, testis, and lungs. PCFT has increased expression in the small intestines, primarily the duodenum.

PCFT has high activity at low pH, decreasing to about one-fifth its activity at neutral pH. PCFT contrasts RFC, which at lower pH showed a decreased affinity for folate and an increased affinity for antifolates. PCFT activity increases at lower pH due to a greater current. Using the low pH (high hydrogen ion concentration), the electrochemical gradient pushes folate and hydrogen ions both across the membrane.

Beyond the membrane potential, transport is also sensitive to the folate gradient. As that gradient increases, transport increases. At low membrane potentials, the current can reverse and become negative, reflecting the flow of the negative folate molecule. The transporter also has channel-like activity. At very low pH, the transporter allows protons across alone.

Mutations in the enzyme can disrupt enzyme function. The Glu185 residue is critical to proton coupling. The His281 residue appears to play an important role in proton binding. When it is mutated, there is decreased affinity for folate substrates. In addition, Asp109 is required for function. When mutated, the carrier is inactive, irrespective of charge or polarity. These mutations have detrimental effects on folate absorption and reabsorption, where PCFT is the primary transporter.

Folate Transport and the Placenta

All three folate transporter types are present in the placenta. There is a high level of low-pH folate transport activity, with a lower level of activity at neutral pH, indicating large PCFT to RFC ratio.

Folate Homeostasis and Absorption

Folate homeostasis is determined by the amount of folate in the diet, the efficiency of intestinal absorption, folate in circulation, and folate retention. Folate deficiency results in increased expression of RFC, PCFT, and FRα in the small intestine and kidney. This maximizes absorption and reabsorption. In addition, other folate receptors are shut down to conserve folate for important tissue, like brain, heart, placenta, and lungs.

Folate and Neural Tube Defects

Chronic folate deficiencies during pregnancy cause a range of disabilities.
known as neural tube defects (NTDs). In this case, the neural tube, which encases spinal and brain tissue, fails to close during gestation. Left open and exposed, it leads to conditions such as spina bifida and anencephaly\textsuperscript{13,14}.

Folate mediates neural tube closure via the already discussed folate cycle. Disruption or lack of THF leads to neural tube defects.

More specifically, scientists have found that decreased B9/B12 levels in women who have had an affected pregnancy are lower than those who do not have affected offspring, even under the same folate supplementation\textsuperscript{14}. This suggests deficiency in the folate cycle of the mother or offspring, or a down-regulated transporter. Several trials using folic acid supplementations have proved successful at preventing neural tube defects\textsuperscript{13,15}, indicating that the possible problematic enzymes are under-regulated rather than non-functioning.

\textit{Mother/Offspring Enzyme deficiency}

Research has discovered more than 40 mutations in the MTHFR gene associated with decreased or inactive enzyme function. The most common is the single nucleotide polymorphism (SNP) known as 677C>T or the 677\textsuperscript{th} position in the MTHFR gene changes from the cytosine wildtype to a thymine. Individuals with this allele have severely decreased MTHFR function. Levels of methionine and activated SAMe decrease while toxic levels of homocysteine increase. Mothers with the 677C>T gene are more likely to have offspring affected with NTDs because they are unable to share enough folate with the child. This particular state can be identified by mother health, as low or average levels of folate may be insufficient for normal health and symptoms of hyperhomocysteinemia are present\textsuperscript{16,17}.

Currently mothers supplement their diet with excess folate or folic acid, thereby over-saturating the enzymes and producing maximum MTHF while having excess folate for the offspring\textsuperscript{18}.

\textit{Transporter deficiency}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{Figure3.png}
\caption{Common Neural tube defects (NTD).}
Spina Bifida (left) is where the neural tube doesn’t close along the spine, Anencephaly (middle) where the neural tube doesn’t close near the top of the skull, resulting in parts of the brain and skull missing. Encephaloceles (right) where the neural tube does not close, forming a sac due to part of the brain. Figure from Google images.
\end{figure}
Another cause of NTDs is the inability for folate to pass from the mother to the child during gestation. Transporters across the syncytiotrophoblast may be impaired, through either genetics or regulation.

There has been research that states a nutrigenomic relationship between folate and folate transporter along placenta tissue. In individuals with hyperhomocysteinemia or NTD-affected children, there is methylation of the folate transporter activation gene RFC1 (reduced folate carrier 1), causing decreased folate transport\(^1\). This is likely a survival mechanism in the presence of low folate. In addition, suppression of PCFT decreases overall folate levels in circulation, resulting in decreased expression of RFC1\(^1\). As mentioned, allelic suppression of RFC or FR transporters results in NTD-affected offspring and in some cases fetal death\(^1\).

In addition, regulation may cause a decrease in folate transport. Due to SAM’s functions with DNA and RNA methylation, folate is a required nutrient in new dividing and differentiating cells. Therefore, folate and its transporters are heavily regulated\(^9\).

In either case, the current methodology for overcoming decreased folate transport is to super-saturate the transporters thereby causing maximum output\(^1\). Via folate supplementation, Spina bifida rates have decreased in the United States from 5/10000 to 3.5/10000\(^1\).

### Folate and Cancer

Folate and cancer have a complex relationship. Many studies have verified that the supplementation of folate decreases the risk of cancer formation. This is thought to occur due to folate’s synthesis of thymidylate and purines and DNA methylation via SAM\(^20-22\). However, cancer cells, which rapidly grow and divide, require folate to catalyze nucleotide synthesis. Studies have shown that expression of folate receptors are increased in related cancers such as epithelial cancer and leukemia\(^1\). Other studies have found that cancer cells up-regulate all 3 types of folate transporters on their surface, allowing them to multiply faster\(^21\). So although folic acid has been shown to significantly decrease the risk of many cancers\(^20-23\), it has also been shown to increase the progression of already present cancer\(^21\). Even this is dependent upon the form and amount of folate supplementation. Synthetic folate, or folic acid, in low doses decreases progression of cancer. In high doses, it increases cancer progression. In contrast, natural folate does not seem to affect cancer progression at all. This is thought to be caused by the increased bioavailability of folic acid making it more potent\(^21\). In addition, some transporters, especially on cancer
cells, have shown a preference for folic acid over folate\textsuperscript{20,21}.

Although studies have connected folate to prevention and progression of gastrointestinal cancers, a recent study found a correlation between decreased risk of breast cancer and adequate folate levels\textsuperscript{21}, therefore making it cancer preventative. If breast cancer follows the trend of gastrointestinal cancers, there is a strong probability that over-supplementation of folic acid would increase the progression of breast cancer. Progression of cancer, especially breast cancer, is of high concern to women due to the increased folate supplementation during pregnancy. Therefore, folate supplementation may not be the best course of action to overcome insufficient folate transport. Changing the factors that affect regulation will cause less risk.

**Folate Transport Inhibition by Autoantibody**

As previously mentioned, folate is a heavily regulated nutrient\textsuperscript{12,14,16,21,24,25}. Cells that are rapidly growing, dividing, or replicating such as infected or cancerous tissue require folate. These kinds of cells trigger the immune system, which may play a role in the regulation of folate transporters\textsuperscript{26}.

**Autoantibodies**

Antibodies, soluble, antigen-binding immunoglobulins (Ig), are major regulators within the immune system. These proteins are produced by B cells, part of the adaptive immunity. The Ig works by binding to an antigen and signaling for destruction\textsuperscript{26}.

During initial differentiation B cells go through clonal selection. If the cell’s receptor (BCR) binds to self-antigens, then

![Figure 4. Immunoglobulin (Ig) isotypes\textsuperscript{4}.](image)

When a B cell terminally differentiates, it undergoes receptor editing and isotype switch. The membrane-bound IgM BCR proteins become a soluble IgM pentamer, a soluble IgA dimer, or soluble IgE or IgG monomers. IgD is typically only bound to B cell membranes. These isotypes are specialized at fight specific types of infections.

the B cell is destroyed. In theory, all B cells are non-self reacting. However, some B cells slip past the selection process, reacting to proteins on self-cells. In addition, during the receptor editing stage of terminal maturation into a plas-
Plasma cell, the B cell can become self-binding. B cells undergo final maturation only if they receive helper T cell cytokine signaling. To release cytokines a T helper cell must bind an antigen-presenting cell's (APC) MHCII receptor. In this case, the antigen has been ingested, digested, and a fragment put on the surface. Although T cells undergo selection against self, some still react to self-sequences and are called regulatory T cells. Activation by T cells does more than signal for maturation, it also up-regulates the entire inflammatory process. This leads to a more acute response at the sight of the antigen.

One of the benefits of B cell maturation into a plasma cell is receptor editing and isotype switch. Receptor editing is the process by which the antigen-binding site of the BCR is optimized to bind stronger. Isotype switching change the Ig protein that make up the BCR is fundamentally changed. Different isoforms include IgA, IgD, IgE, IgG, and IgM. The standard Ig is IgM. However during maturation it can become one of the other ones. These isoforms are specialized for different pathogens and situations. IgG is very good against viral and bacterial infections. IgE is good against fungal and parasitic infections. Finally, IgA is used in secretions like breast milk or mucous.

Even without T cell cytokine signaling, the B cell can still mature and differentiate, although not into a full plasma cell with a specialized immunoglobulin protein. Because no isotype switching and receptor editing of the BCR occurs in this type of differentiation, the Ig produced have weaker bonds and are therefore less potent. In addition, without T cell pro-inflammatory cytokines, the response itself is quite weak.

Antibodies produced that react to self are known as autoantibodies. Although they arbitrarily kill self-cells, they can be beneficial. They are the body’s mechanism to destroy cancerous cells and hard-to-find infections.

**Autoantibodies as receptor disruptors.**

The FR folate transporters are highly antigenic. Increased expression of folate receptors evokes an autoantibody response in high susceptible patients, like those with breast or ovarian cancer. In addition, autoantibodies can work two ways. Antibodies can bind to the receptor, turning the enzyme off, or they can block the active site. Both methods can disrupt the uptake of folate into the cell.

The effectiveness of autoantibodies in disrupting folate transport can be seen in cerebral folate deficiency (CFD). CFD is caused by folate receptor autoantibodies (FRAs). These disrupt the transport of folate across the epithelial lining of the blood-brain barrier. Supplementation with folic acid improves some of the effects of CFD including verbal communication, receptive and expressive language, attention and stereotypical behavior.
Maternal autoantibodies can also disrupt similar folate receptors on the placenta border. Folate must leave the bloodstream of the mother, entering the intervillous space. From here, folate must enter the fetal bloodstream via the umbilical cord. Several layers of epithelial tissue surround fetal capillaries and veins in the placenta: the syncytiotrophoblast, the cytotrophoblast, and the mesoderm. All three transporters mediate this entire process. First, the folate must enter the intervillous space that is primarily regulated by RFC transporters. From here it must cross the syncytiotrophoblast and cytotrophoblast. It is known that FRα is instrumental in regulation across the syncytiotrophoblast and the other layers of epithelial tissue. Finally, PCFT transports have been theorized to be on the epithelial border for folate absorption. Although all three types of transporters exist in the placenta, the primary transporter across epithelial tissues is the FRs. Several studies have found that binding inhibition of FRα has been correlated with an increased risk of NTDs. Furthermore, in 2008, the presence of IgG and IgM antibodies to FR and inhibition of folic acid binding to FR has been found to be a risk factor for NTDs. The presence of IgG is interesting because it infers an isotype switch and receptor editing has occurred within the autoreactive B cells. Therefore, T cell activation has taken place. The presence of IgG in only some of the patients may explain the range of acuteness of symptoms in offspring.

However, contradictory data was found in 2009, which stated that there was no statistical difference in FRα antibody titer (percentage of antibody in serum) between the non-affected and affected in their studied population. However, they assert that there is still the possibility that increased titer of autoantibodies in early pregnancy may exacerbate the effect of low folate levels and thereby influence the outcome of the neural-tube defect.
In addition, the function may not be directly correlated to the single studied receptor. Two other transporter types exist, both on the border, which mediate folate to the fetus. These supplement folate disruptions in FRα. Further research is needed to determine what kinds of antibodies, how much, and in what receptors lead to the higher risk of NTDs.

**Autoantibody Mediation by Omega-3 and Omega-6**

One possible mediator of autoantibodies is the omega-3/omega-6 ratio. Omega-3 fatty acids have long been associated with decreases in inflammation and deactivation of the adaptive immune system via down-regulation of helper T cell cytokine output\textsuperscript{32}. Omega-6 fatty acids have been associated with pro-inflammatory signaling via eicosanoids\textsuperscript{33}. Both omega-3 and omega-6 fatty acids become eicosanoids through enzymes known as COX-1 and COX-2\textsuperscript{34}. In addition, LOX plays an important role in inflammation maintenance via polyunsaturated fatty acid eicosanoids\textsuperscript{35,36}.

**COX-1, COX-2, and LOX**

Cycloxygenase 1 (COX-1) and Cycloxygenase 2 (COX-2) are enzymes that convert polyunsaturated fatty acids into powerful hormones called eicosanoids. Studies in both COX-1- and COX-2-

![Figure 6: omega-3 and omega-6 products via the COX and LOX pathways\textsuperscript{4}.](image)

As can be seen here, the omega-3 products are primarily anti-inflammatory. The omega-6 products are primarily pro-inflammatory. However, at the correct omega-3 to omega-6 ratios, the products are more anti-inflammatory than omega-3 alone.
knockout mice reveal impaired inflammatory responses, although with varying intensity. Furthermore, COX-1 and COX-2 knockouts have decreased symptoms of arthritis, an inflammation related disease. In addition, a human trial shows that COX-1 products drive the initial phase inflammation, with COX-2 occurring within several hours.

The COX enzymes work by adding O2 as peroxide linkages to a polyunsaturated fatty acid (PUFA). A carbon ring forms near the center. An oxygen is released, forming the toxic reactive oxidative species and the eicosanoid.

COX-1 is constitutive and produces protective prostaglandins (PG), a subfamily of eicosanoids, which aid in homeostatic functions, like vasodilatation, gastrointestinal movement, platelet formation, and macrophage differentiation. There is evidence that COX-1 is activated by LPS-mediated inflammation (LPS being a major bacterial antigen). This might explain the pro-inflammatory correlation sometimes seen in human and mice trials.

With omega-6s, COX-2 is strongly pro-

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**Figure 6**: Fate of Arachidonic Acid (AA) in COX and LOX pathways.

The LOX pathway for AA is in green. Blue depicts the pathway of COX-1 and COX-2. The products of these pathways, leukotriene (LT) and prostaglandins (PG) are both strong anti-inflammatory eicosanoids.
inflammatory. It is regulated on endothelial tissue and produces two types of pro-inflammatory factors: PGs and prostacyclins. These cause fever, pain sensitization, platelet aggregation, and cell differentiation. However, in the presence of omega-3s, COX-2 produces anti-inflammatory eicosanoids called electrophilic oxo-derivatives (EFOX). This activates PPARγ that suppresses the release of pro-inflammatory cytokines.

Lipoxygenase (LOX) uses 5-lipoxygenase activating protein (FLAP) to catalyze arachidonic acid (AA), an omega-6, into 5-hydroperoxyeicosatetraenoic acid (5-HPETE). This reduces to 5-hydroxyeicosatetraenoic acid (5-HETE) and LTA synthase acts on 5-HETE to convert it into leukotriene A (LTA). Other forms of leukotrienes (LT) are formed from a series of intracellular and extracellular enzymes. In the presence of omega-3s, LOX will produce resolvins (RvEs). Even small amounts of omega-3 will cause a shift from pro-inflammatory to anti-inflammatory factors. However, both are required for full anti-inflammatory function, indicating a synergistic relation between the two compounds.

**Omega-3 and inflammation**

Docosahexaenoic acid (DHA) and other omega-3 PUFAs act as anti-inflammatory agents. They do this via

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**Figure 7: Major omega-3 and omega-6 polyunsaturated fatty acids (PUFAs).**

The primary dietary form of omega-3 is α-linolenic acid (ALA). Biologically active forms are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The primary dietary form of omega-6 is linoleic acid (LA). The biologically active form is arachidonic acid (AA).
three different methods 1) changing mesodomains on the cell membrane, 2) hormonal regulation, and 3) resolin and protectins (eicosanoids from COX-1 and COX-2).

Omega-3 PUFAs become integrated into the cell membrane, increasing fluidity. The concentration and type of different lipids determines the fluidity of the cell surface. Mesodomains and lipid rafts are parts of the membrane with organized and distinctive structure. They are usually composed of sphingolipids and cholesterol, making them much less fluid than the surrounding membrane. Typically, these mesodomains bring all the necessary proteins together so that signal transduction can occur. For instance, in helper T cell (T\(_H\)) activation, there is a T cell receptor, which must bind to MHCII. To become fully activated the CD4 on the T cell must also bind to the MHCII receptor. By changing the lipid composition of the membrane, i.e. increasing the omega-3 PUFAs, mesodomains and lipid rafts can be altered. If the mesodomain containing these proteins becomes more fluid, than there is less probability that these proteins will be adjacent when the T\(_H\) cell encounters an APC and therefore less probability of activation.

Research has shown that DHA modifies the lipid membrane on T helper cells, therefore decreasing T helper cell. It does this by targeting and Acyl-CoA binding protein as well as activation proteins. 

DHA and other omega-3 PUFAs can act directly as hormones. PUFAs, being fats, can bind surface proteins but also pass through the membrane to bind intracellular or nuclear receptors. One major gene product affected by DHA is NF-κB. NF-κB is responsible for the activation of COX-2, nitric oxide synthase, and a host of pro-inflammatory cytokines. Two cytokines, IL-6 and IL-12, up-regulate T\(_H\) cells, which in turn produce II-4 and II-5 that up-regulate B cells. In the case of NF-κB, DHA activates transcription factor PPARγ. PPARγ competes with NF-κB, which was activated via a toll-like receptor (TLR) on the surface of the APC cell. NF-κB is down-regulated, therefore down-regulating the other pro-inflammatory factors.

Finally, eicosapentaenoic acid (EPA) and DHA up-regulate the production of resolvins and protectins in the LOX and COX-2 pathway to decrease an active immune response. These work by binding to immune cells and chemicals and decreasing migration to immune site.

Omega-6

Omega-6 PUFAs are especially potent anti-inflammatories via the action of AA. AA is converted into PGs via the COX-2 pathway, as already discussed. PGs then act upon neurons, endothelial tissue, and macrophages to increase the inflammatory-
ry response. This increases the autoantibody response, which would decrease targeted enzyme efficiency.

**Omega-3 to Omega-6**

Research has found that high levels of omega-3s are detrimental because they suppress the immune system against pathogens. In addition, too much omega-6 is correlated with an overly-reactive immune system, in which inflammation occurs in the absence of a pathogen.

The current western diet is heavily skewed towards omega-6. This is due to the decreased consumption of omega-3 type foods (fish like salmon, etc.) and increased consumption of omega-6 rich foods (oil, grains, grain-fed cattle, etc.). This shift mimics the shift from anti-inflammatory to pro-inflammatory conditions like arthritis, allergies, and eczema.

Studies have shown that the ideal ratio of omega-3 to omega-6 is 1:1 and not to exceed 1:4. This recommendation has been backed by the National Institutes of Health. Diet outside of these ratios may lead to poor or lower performing health because the immune system is not balanced for reaction and over-reaction to pathogenic material. The current western diet sits at ratios from 1:10 to 1:25.

**Autoantibody Mediation by NSAIDs**

COX-1 and COX-2 are targets of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs compete with AA and DHA for the active site of these enzymes. Therefore, use of NSAIDs will decrease inflammation (and through that pain sensitization). Ultimately, this might be another method of decreasing pro-inflammatory eicosanoids, thereby decreasing autoantibody production.

**Summary of Autoantibody Inhibition**

Folate transport can be decreased by autoantibodies. Autoantibodies are produced by B cells in response to self-antigenic stimulation. T cell cytokines up-regulate this activity. COX-1, COX-2, and LOX play an important role in increasing inflammation and therefore increasing T cell cytokine and B cell autoantibody output. DHA and EPA are powerful anti-inflammatories by decreasing T helper cell activation, decreasing inflammatory eicosanoids, and increasing inflammatory resolving molecules (resolvins). By decreasing omega-6 and increasing anti-inflammatories like omega-3 and NSAIDs, overall autoantibody titer should decrease. This in turn should up-regulate the affected transporter enzymes thereby increasing folate transport.

**Proposed Methodology**
An epidemiological study should be performed to test the theory that omega-3s decrease the inhibition of folate transport, thereby increasing transport of folate to the fetus.

There are three main premises: 1) Folate deficiency causes birth defects, 2) Folate transporters are mediated by inflammation, 3) Inflammation can be mediated by the omega-3/omega-6 ratio. Largely the scientific community supports the first premise. The scientific community, due to conflicting studies, currently debates the second premise. The third premise is debated by the scientific community, as it is unknown to what extent omega-3 and omega-6 will affect the mediation of autoantibodies.

These premises will be tested using cohort data to determine relationships between folate uptake, omega-3s, and birth outcome. The experiment should consider EPA and DHA independently, omega-6, the amount and method of folate uptake, and the presence of aspirin (NSAIDs). For offspring, head circumference, weight, health status, and birth defect status should be noted for overall outcome. Because of the multiple factors, the statistical work up with ANOVA would be appropriate. Finally, individual interactions should be observed for correlations. Expected outcomes:

- Offspring outcome should positively correlate with folate.
- Offspring outcome should positively correlate with DHA.
- Offspring outcome should positively correlate with EPA.
- Offspring outcome should negatively correlate with omega-6.

The main control of this experiment is the correlation of folate and NTD. If the correlation does not exist, it might be faulty data or a faulty primary premise. The second control is the relationship of CFD to omega-3s. CFD is largely mediated by FR autoantibodies. On the other hand, NTD folate deficiency is more complex, and may prove inconclusive. However, a correlation between CFD severity and omega-3 intake, as theorized, would indicate the possibility of the third premise, lending support to the overall hypothesis that healthy omega-3/omega-6 ratios increase folate transport to the fetus.

Should the data suggest a positive correlation between DHA and beneficial offspring outcome, the next step will be a mouse model. Due to the contention of autoantibodies decreasing folate transport, titer of autoantibodies in the placenta should also be measured. CFD, a condition caused by low folate transport, should also be used in this study as a control.

**Program**

To test our hypothesis, we used the NBDPS database provided by the CDC.
NBDPS provides diet and child outcome for more than 15000 mothers/child sets. The USDA nutritional database provides the amount of folate, DHA, EPA, and ω-6 in the foods appearing in the mothers’ diets. As these two tables are not in the same database (and one is illegal to copy or modify), it is impossible to get the information we want with a single SQL query. Therefore we queried for all of the foods consumed by each mother as well as the birth outcomes from the first database. We then queried the USDA database to replace subjects’ diets with the amounts of DHA, EPA, or ω-6. From there, it would have generated graphs relating each concerned substance to each measure of birth outcome (16 graphs total). Graphs of NSAID usage versus each birth outcome (4 graphs) could be generated after a single SQL query since the second database is not needed as the first contains drug usage explicitly.

Both of these databases were created with an ancient version of Microsoft Access. Using the program is problematic on modern operating systems since some of the functionality is no longer supported. In addition, accessing the data programmatically without requiring the program to run on a Windows operating system would usually require a specialized ODBC driver (Microsoft provides such a driver for Windows, though configuring it is non-trivial). Luckily, an open source Java library called “jackcess” provided by Health Market Science, Inc. provides programmatic access to Microsoft Access databases through the Java programming language.

Unfortunately, we did not have permission to access the birth outcomes recorded for each case. With no way of determining birth outcomes, no relationship between any substance and birth outcome can be determined.

**Conclusion**

Folate deficiency in the fetus leads to the devastating effect of neural tube defects. Inhibition of folate transporters can lead to CFD during early childhood. To counteract both of these diseases, supplementation with folate is suggested. However, with the recent discovery that folate increases the progression of cancer, it may not be an advisable treatment in all cases. Instead, supplementation aimed at decreasing inhibition of the folate transporter may prove useful. This is especially true of NTD, in which mothers are at a high risk for breast cancer, but also in the treatment of CFD, which has strong correlation between affliction and anti-folate antibody titer. Epidemiological studies are a good next step with mouse studies to follow should there prove to be a correlation. Successful treatment of these conditions would not only improve welfare but also enrich the current understanding of autoantibody inhibition.
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