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Review

Consequences of dietary methyl donor supplements: Is more always better?

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ABSTRACT

Epigenetic mechanisms are now recognized to play roles in disease etiology. Several diseases increasing in frequency are associated with altered DNA methylation. DNA methylation is accomplished through metabolism of methyl donors such as folate, vitamin B12, methionine, betaine (trimethylglycine), and choline. Increased intake of these compounds correlates with decreased neural tube defects, although this mechanism is not well understood. Consumption of these methyl donor pathway components has increased in recent years due to fortification of grains and high supplemental levels of these compounds (e.g. vitamins, energy drinks). Additionally, people with mutations in one of the enzymes that assists in the methyl donor pathway (5-MTHFR) are directed to consume higher amounts of methyl donors to compensate. Recent evidence suggests that high levels of methyl donor intake may also have detrimental effects. Individualized medicine may be necessary to determine the appropriate amounts of methyl donors to be consumed, particularly in women of child bearing age.

Contents

1. Introduction

Epigenetic mechanisms have been shown to play roles in disease etiology. Thus, it is now widely believed that genetic and environmental factors act in tandem via epigenetic mechanisms to underlie many diseases (Altobelli et al., 2013). Epigenetic modifications are now widely considered the missing heritability in diseases that do not follow traditional Mendelian inheritance patterns (Portela and Esteller, 2010; Robertson and Wolffe, 2000). For example, epigenetic effects such as genomic imprinting cause more complicated pedigrees and thus epidemiology (Handel et al., 2010).

Environmental factors altering epigenetic patterns include maternal diet, stress, radiation exposure, infectious agents, and immunological factors (McGowan et al., 2008; Verma, 2003).

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Dietary effects on epigenetic status have been of particular interest since Barker and others have proposed that low protein levels during human development may predispose to specific diseases later in life (Barker, 1992, 1993; Wadhwa et al. 2009).

Folate and related B vitamins contribute to the carbon/methyl donor pathway, critical for addition of epigenetic marks to DNA and proteins, among other functions. Maternal folic acid consumption has been strongly linked with reduction in the frequency of neural tube defects (Honeinm et al., 2001). This association led to the U.S. mandating fortification of grains and cereal in 1998 (Bailey and Gregory, 1999).

Folate (as Folic acid, FA) and other methyl-donor pathway components are now added to a variety of consumables. For example, vitamin B12 is a common additive in energy drinks, and betaine/trimethylglycine (TMG) is used to reduce homocysteine levels (Wang et al., 2013). Products in this pathway are marketed as health products and for their ability to promote methylation (e.g. http://www.olaloa.com/ola-loa-and-the-healing-power-of-methylation).

Several diseases have increased in frequency within this post-fortification time frame, leading to speculation that increased methylation may contribute to their etiology (Barua et al., 2014a; Hollingsworth et al., 2008; Kim, 2007; Van den Veyver, 2002). These diseases include cancers, neurological disorders, growth syndromes, respiratory disorders, and multiple sclerosis (Dominguez-Salas et al., 2012; Portela and Esteller, 2010; Schaevitz and Berger-Sweeney, 2012; Schanen, 2006; Sharp et al., 2013; Skinner, 2011). In particular, autism spectrum disorders (ASD) have increased from 1 in 250 in 2000 to 1 in 68 in 2013 (Schenkelberg et al., 2014). A possible connection between this increase and increased intake of methyl-donor pathway components is being hotly debated (Neggers, 2014; Schaevitz and Berger-Sweeney, 2012).

Dietary methyl-donors were first shown to have epigenetic effects in classic animal studies which showed that high maternal intake can silence retroelement induced mutations at the agouti A^{vy} allele and the similar $Axin^{Fu}$ allele (Cooney et al., 2002; Waterland et al., 2006a; Waterland and Jirtle, 2003; Wolff et al., 1998). The diet used in these studies has also been shown to reduce the effects of de-methylating agents such as BPA (Dolinoy et al., 2007) and mitigate some effects of a high fat diet (Cordero et al., 2014). In addition, reduced amounts of dietary methyl donors have been shown to have negative effects (Altobelli et al., 2013).

Several studies have suggested more broad effects of these supplements on the methylome. In addition, folic acid fortification has been associated with an increase in DNA methyltransferase (DNMT) activity (Ding et al., 2012). Consequently, a growing number of recent studies suggest deleterious effects of developmental exposure to high doses of methyl-donor pathway components (Barua et al., 2014a, 2014b; Mikael et al., 2013; Shorter et al., 2014; Vasquez et al., 2013).

Alterations of methyl donors in the maternal diet have been associated with altered methylation at several genes and aberrant behavior phenotypes (Barua et al., 2014a). In this review, we focus on the broad potential effects of altered methyl donor levels in both maternal and post-weaning diets in animal and human studies, with specific discussion of some ASD relevant data. Notably, we spend little space on deficiencies of these molecules. We suggest genetic variation (both known and novel alleles) will ultimately be seen as a major factor in recommendations for dietary intake of these molecules.

2. Key components of the methyl donor pathway

Dietary supplements that increase the level of methyl donor pathway components include FA, cobalamin (Vitamin B12), choline, betaine/trimethylglycine (TMG), and L-methionine. Methionine metabolism is regulated by nutrient intake of B12, pyridoxine (Vitamin B6), and riboflavin (Vitamin B2) since these B vitamins are cofactors for hormones that regulate methionine metabolism (Kalhan and Marczewski, 2012). Methionine is the precursor molecule to S-adenosylmethionine (SAM) which actively donates a methyl group to more than 60 products including DNA, RNA, and histones (Hollenbeck, 2012). Choline is oxidized to betaine aldehyde by betaine aldehyde dehydrogenase, which is a niacin (Vitamin B3) dependent enzyme (Hollenbeck, 2012). Synthesis of betaine/trimethylglycine from choline is an irreversible process in humans. TMG, FA, and B12 are important coenzymes for conversion of homocysteine to methionine.

The folate metabolism pathway leads to purine synthesis and synthesis of 5-methyltetrahydrofolate (5-MTHF) using the enzyme 5-methylenetetrahydrofolate reductase (5-MTHFR) and a cofactor Vitamin B2 (Fig. 1). Methionine is then produced with assistance from Vitamin B12 and methionine synthase (MS). Methionine is converted to S-adenosylmethionine (SAM), the methyl donor molecule. SAM donates a methyl group while methyltransferase enzymes add the methyl group to DNA, RNA, proteins, and lipids.

3. Methyl donor components in supplements and fortified food/beverages

Fortification of grain products with FA began in the United States and other countries in the 1990s, as FA consumption was correlated with decreased neural tube defects (NTDs) (Berry et al., 1999; CDC, 1991; Czeizel and Dudás, 1992; Honeinm et al., 2001; Godwin et al., 2008; Vanhees et al., 2014) and a decrease in low birthweights (Timmermans et al., 2009). While spina bifida and ostium secundum atrial septal defects were both decreased post fortification, there were increases in urinary tract obstructive defects and increased pyloric stenosis (Godwin et al., 2008). Folate/FA deficiency is linked to anemia, atherosclerosis, NTDs, adverse pregnancy outcomes, psychiatric disorders, and cancers (Bailey et al., 2003; Brito et al., 2012; Giovannucci, 2002; Reynolds, 2014), but FA intervention trials in humans are inconsistent and are not completely supportive of protective effects of FA supplementation except in the case of NTDs (Bønaa et al., 2006; Clarke et al., 2010; Lonn et al., 2006).

Therefore, it has been questioned whether extra folic acid through food fortification is really beneficial to the majority of the population (Smith et al., 2008). Foods were fortified in the United States beginning in 1996 after the FDA approved fortification of grains at a dose of 140 ug FA/100 g of food to place approximately 100 ug FA more into the average adult diet (Table 1) (Hoyo et al., 2011a). Trials around the world have documented increased serum folate concentrations after foods were fortified with FA (Johansson et al., 2002; Neuhouser et al., 1998; O'Keefe et al., 1995; Tucker et al., 2004) or after supplementation with FA (Brouwer et al., 1999; Hao et al., 2008; Houghton et al., 2011; Hursthouse et al., 2011; Neuhouser et al., 1998; Venn et al., 2002). Notably, FA added to foods during fortification is 70–85% bioavailable (as folate) compared to only 50% bioavailability of FA/folate naturally occurring in foods (Hoyo et al., 2011a; Quinlivan and Gregory, 2007; Winkels et al., 2007). Note that it is commonly assumed that the maternal and fetal genotypes respond similarly to the recommended dosages.

FA supplements and FA fortified foods are recommended to women who may become pregnant (Hoyo et al., 2011a). Therefore, if a mother consumes both supplements and fortified foods during pregnancy, it is possible both mother and fetus may be exposed to amounts of FA that exceed the recommended tolerable upper limit of 1000 ug/day for adult pregnant women (Hoyo et al., 2011a). Diets

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