LONGEVITY MEDICINE REVIEW™
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Beyond the Mitochondrial Tune Up: Part II

by Lara Pizzorno, MDiv, MA, LMT

Part II: The Methylation – Transsulfuration Connection to Mitochond

Introduction

Mitochondrial decay resulting from oxidative damage accumulates with age and is universally recognized as a major contributing factor to the whole range of functional decline and tissue deterioration associated with aging.[1][2] [3] [4] [5] [6] [7] Part I of this review discussed Bruce Ames' application of the Michaelis constant (KM) concept to the ramifications of age-associated oxidative damage to proteins. Specifically, with age, increased oxidative damage to key enzymes produces deformation in their structure, resulting in an increased Michaelis constant (KM), i.e., a decreased binding affinity for the co-enzyme (the nutrient co-factor for the enzyme), thus causing a decrease in enzyme function.[8] Ames' research has demonstrated that increasing the availability of acetyl L-carnitine, the substrate for the enzyme acetyl-L-carnitine transferase, (which plays a key role in transporting long-chain fatty acids into the mitochondria for β *oxidation* and ATP production), along with α-lipoic acid, (a mitochondrial antioxidant that also serves as cofactor for two key enzymes in the Krebs cycle, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase), restores the velocity of the reactions (KM) for these enzymes, and thus restores aging mitochondria's ability to regain youthful levels of energy production.[9] [10] [11] [12] [13]

Single Nucleotide Polymorphisms plus KM equals Double Jeopardy

In addition to the aging-associated accumulation of oxidative damage, single nucleotide polymorphisms (SNPs) are another factor affecting KM, and one that, since the activity of the enzymes affected is down-regulated from birth, may promote premature aging. As many as one-third of all mutations in a gene result in the corresponding enzyme having an increased KM (decreased binding affinity) for a coenzyme, and therefore a lower rate of reaction.

One well-researched and pivotal example in terms of its impact on mitochondrial function is the 677CàT SNP of methylenetetrahydrofolate reductase (MTHFR), a key enzyme in the methylation cycle. MTHFR is responsible for the conversion of folic acid into methionine synthase, which, along with B12, recycles homocysteine to methionine.

In the fairly common 677CàT variant (~30-40% incidence), thymine is substituted for cytosine in the gene, resulting in production of a slow variant of the MTHFR enzyme in which valine is substituted for alanine at position 222 (for which reason this SNP is also labeled Ala222Val). In individuals in whom both alleles have this SNP (i.e., individuals homozygous for the TT genotype of MTHFR, (~10% of the North American population[14]), the resulting enzyme expressed has only 50% of the activity of the wild (more common) CC type.[15]

Similar to the way in which providing supplemental acetyl-L-carnitine restores the KM for acetyl-L-carnitine transferase, providing supplemental folate, the substrate for MTHFR, may help compensate for the drop in MTHFR activity that will otherwise occur in carriers of this SNP. Another way of looking at this is that carriers of the MTHFR 677CàT SNP have an increased requirement for folate to optimize the activity of this enzyme, and thus require more folate for normal methylation.

Whether impaired MTHFR activity is the result of age-associated oxidative damage or genetic inheritance, restoring normal levels of MTHFR activity is essential for optimal function in aging mitochondria. When MTHFR is compromised, not only is methylation impaired, but unmetabolized homocysteine accumulates in the cell and effluxes into the bloodstream. In both, homocysteine will react with reactive oxygen intermediates, greatly increasing the rate of antioxidant consumption, including that of GSH, an antioxidant critical for neutralizing mitochondrial reactive oxygen species (ROS).

High levels of homocysteine have been shown to increase ROS and lipid peroxidation in the endothelium, liver, kidney and brain.[16] [17] [18] [19] In addition to decreasing antioxidant defenses and total *thiol* content (Thiols are compounds that contain the functional group [–SH], aka a sulfhydryl or thiol group. [–SH] is the sulfur analogue of an alcohol group [–OH]. Key compounds in mitochondrial energy production, coenzyme A, cysteine, GSH, and α-lipoic acid are all thiols), hyperhomocystenemia has been shown to disrupt the activity of all three key mitochondrial antioxidant enzymes (superoxide dismutase [which neutralizes superoxide radical ($O2^-$)], catalase [which neutralizes hydrogen peroxide ($H2O2$)] and glutathione peroxidase (GPx) [which reduces $H2O2$ and lipid peroxides]).[17]

Homocysteine Metabolism - Linking Methylation, Transsulfuration and Mitochondrial Function

Decreased MTHFR activity hamstrings the methylation cycle, resulting in increasing levels of homocysteine, increased ROS production, decreased S-adenosylmethionine (SAM) production, and increased oxidative stress, which greatly increases the rate of glutathione (GSH) depletion.

In addition to its role in the methylation cycle, homocysteine is involved in transsulfuration as the precursor to cysteine, the rate-limiting factor for the production of GSH, the premier endogenous antioxidant. Thus homocysteine serves as an essential factor in the body's major antioxidant system—*when other processes utilizing homocysteine, i.e., methylation, are functioning normally*. However, when the interactive balance among these pathways is compromised, alteration in the level any of the thiols affects the others.[20]

Compromised methylation results in increased homocysteine efflux into plasma, where it is highly susceptible to being oxidized, increasing the formation of superoxide radical (O2−) and hydrogen peroxide (H2O2), thus increasing systemic demand for GSH. When methylation is chronically compromised (as in an individual homozygous for the 677CàT SNP or one with insufficient intake of nutrient co-factors essential for the activity of enzymes involved in methylation, e.g., vitamins B6, B12, folate and riboflavin), plasma levels of homocysteine are chronically elevated, resulting in chronically increased need for GSH. In turn, low GSH concentrations dramatically accelerate homocysteine oxidation, perpetuating the vicious cycle.[20]

Clinical and epidemiological evidence suggests that a plasma level of homocysteine >15 μmol/l, although only modestly elevated, is associated with vascular disease, a finding that may be linked to homocysteine's effects on thiol balance. In recent years, several studies have shown that moderate hyperhomocysteinemia is associated with premature cerebrovascular, peripheral and coronary artery disease.20 Disruption of the interactive balance among the roles played by thiols, resulting in GSH depletion and mitochondrial dysfunction, may prove to be an underlying factor as important as high cholesterol.

The effects of thiol disequilibrium have also been noted in a recent study of autistic children. In these children, the ratio of plasma S-adenosylmethionine (SAMe) to *S-adenosylhomocysteine* (SAH) was significantly reduced; the mean concentration of reduced GSH, the major intracellular antioxidant and mechanism for detoxification, was significantly decreased; and the oxidized disulfide form of GSH (GSSG) was significantly increased, resulting in a 2-fold reduction in the mean GSH:GSSG redox

ratio. Several metabolic precursors for GSH synthesis were found to be lower in autistic children, suggesting insufficient GSH synthesis. These combined findings indicate a significant decrease in methylation capacity (↓SAM:SAH), resulting in a decrease in antioxidant/detoxification capacity (↓GSH:GSSG) and an increase in oxidative stress (↑GSSG) in autistic children.[21]

In this study, 40 autistic children were treated with 75 µg/kg methylcobalamin (2 times/week) and 400 µg folinic acid (2 times/day) for 3 months.

While the intervention did not alter methionine, SAM, and SAH concentrations significantly (despite the fact that methylcobalamin and folinic acid provide methyl groups for the methylation cycle), concentrations of the transsulfuration metabolites, cysteine, cysteinylglycine, and GSH, increased significantly, and oxidized GSSG levels were reduced to the point that after the intervention, these metabolites were no longer statistically different compared to those in healthy control children. Measures of autistic behavior were assessed by a trained study nurse pre- and post-treatment using the Vineland Adaptive Behavior Scales, and significant improvement was observed after treatment, although scores remained below normal.

The finding that plasma concentrations of methionine and SAM were unaffected (and remained significantly below those in the control subjects) was unexpected because methylcobalamin and folinic acid directly provide methyl groups for synthesis of methionine and SAM, and only secondarily provide metabolic precursors for transsulfuration reactions.

The explanation appears to lie in the fact that the enzymes within the methylation cycle, (methionine synthase, betaine-homocysteine methyltransferase, and methionine adenosyltransferase) are redox-sensitive and are down-regulated by oxidative stress. Under pro-oxidant conditions, this serves to promote preferential utilization of homocysteine for GSH synthesis at the expense of methylation.

Similarly, in addition, cystathionine beta synthase (CBS) contains a heme component, which serves as a redox sensor that upregulates CBS activity under oxidative conditions. This adaptive up-regulation of CBS activity further promotes cysteine and GSH synthesis by irreversibly diverting homocysteine away from methionine remethylation and down the transsulfuration pathway.

Short term, the response of these enzymes to oxidative stress is protective, allocating metabolic resources to quickly restore GSH concentrations and maintain intracellular redox status in response to oxidative challenge.

Chronically compromised methylation, however, leads to increased plasma homocysteine levels, promoting chronic oxidative stress. In a state of chronic oxidative stress, synthesis of methionine and its product, SAM, progressively decline with a key factor being oxidative inactivation of the cobalamin cofactor of methionine synthase. In this way, unresolved oxidative stress can promote precursor depletion and a progressive decrease in cysteine and GSH synthesis.

Ames' KM concept provides an explanation for the positive outcome in this study of children with autism, a condition characterized by unremitting oxidative stress. By restoring enzyme activity, treatment with methylcobalamin and folinic acid lowers homocysteine and rescues GSH synthesis.

The Methylation – Transsulfuration Connection to Mitochondrial Decay

As noted in Part I of this review, GSH is the body's most critical intracellular antioxidant and a key conjugating agent in Phase II detoxification. High levels of homocysteine, which lead to chronic oxidative stress and GSH depletion, gravely impact not only mitochondrial function, but that of every cell and system in the body.

Following the logic chain outlined in this article, it is not surprising that folate insufficiency, and the resultant increase in homocysteine levels, which, if chronic, can lead to a decrease in SAM and GSH, has also recently been recognized to be a significant risk factor for Alzheimer's disease and other dementias, and is strongly associated with depression, with approximately one-third of depressed individuals having frank folate deficiency.[15] [22]SAM is a key donor of methyl groups, which are necessary for the formation of neurotransmitters (e.g., serotonin) and phospholipids that are a component of neuronal myelin sheaths, and cell receptors.[23]

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Methylation pathway: (1)Methionine is converted by methionine adenosyltransferase into the methyl donor S-adenosylmethionine (SAM), which is acted upon by methyltransferase and gives up a methyl group, becoming S-adenosyl homocysteine (SAH). SAH hydrolase converts SAH to homocysteine. (2)Homocysteine can either be remethylated through the folate cycle or go down the transsulfuration pathway. (3) The folate cycle requires the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) and folic acid (which enters the cycle as tetrahydrofolate [THF]), plus the enzyme methionine synthase (MS) and vitamin B12, which is the cofactor [in the form of methylcobalamin] for methionine synthase and uses the substrates 5-methyl-tetrahydrofolate and homocysteine. (4) In liver and kidney, homocysteine is also

remethylated by the enzyme betaine homocysteine methyltransferase (BHMT), which transfers a methyl group to homocysteine via demethylation of betaine to dimethylglycine (DMG).

Transsulfuration pathway: (1)Homocysteine is converted to cystathionine by the enzyme cystathionine beta-synthase (CBS), which requires the cofactor vitamin B6 (pyridoxyl phosphate). Once formed from cystathionine via the action of cystathionine gamma-lyase, cysteine can be utilized in a number of cellular functions, including glutathione (GSH) production and protein synthesis.

Methylation / Transsulfuration / Homocysteine Connection to GSH Depletion: When methylation is compromised, plasma homocysteine levels rise, increasing oxidative stress and the rate at which glutathione stores are depleted. When homocysteine elevation is chronic, redox sensitive enzymes in the methylation cycle (methionine synthase and the methyl transferase enzymes, betaine-homocysteine methyltransferase and methionine adenosyltransferase) are down-regulated, which promotes preferential utilization of homocysteine for GSH synthesis at the expense of methylation. In addition, cystathionine beta synthase (CBS) contains a heme component that is thought to serve as a redox sensor that upregulates CBS activity under oxidative conditions. This adaptive up-regulation of CBS activity promotes cysteine and GSH synthesis by irreversibly diverting homocysteine away from methionine remethylation and down the transsulfuration pathway. Short term, this serves as a protective mechanism, quickly restoring GSH concentrations to maintain intracellular redox status during oxidative stress.Chronically compromised methylation, however, leads to increased homocysteine levels and chronic oxidative stress. This results in oxidative inactivation of the cobalamin cofactor of methionine synthase causing progressive decline in synthesis of methionine and SAM, which ultimately promotes precursor depletion and a progressive decrease in cysteine and GSH synthesis.

Optimal Nutrition for Mitochondrial Function

While it has commonly been thought that Americans' intake of essential micronutrients is adequate, evidence indicates that damage occurs at nutrient levels higher than those known to cause acute deficiency disease and, in some individuals (e.g., those with SNPs resulting in lessened KM), it is obvious that higher than DRI levels of the associated enzyme co-factors are necessary for optimal function.

In addition to folate, MTHFR contains a bound flavin cofactor (derived from riboflavin, which as noted in Part I is a nutrient cofactor also required in the Krebs cycle and electron transport chain) and uses NADPH (which requires niacin as its co-factor and also plays a central role in mitochondrial oxidative phosphorylation) as the

reducing agent. SNPs resulting in an increased need for both riboflavin and niacin have also been identified.

The key clinical take-away, first proposed by Bruce Ames in his seminal paper on SNPs, decreased coenzyme binding affinity, and related nutrient needs, and recently re-emphasized by leading nutrigenomics researcher Michael Fenech, is that the activity of the reaction catalyzed by the MTHFR gene—and therefore the risks associated with the lessened MTHFR activity seen in carriers of 677CàT—can be markedly modified by the providing higher than "average" amounts of not just folate, the substrate for MTHFR, but also riboflavin and niacin, its cofactors.[25] [8]

Basic biochemistry and other studies suggest that not only higher than DRI amounts of riboflavin and niacin, but of vitamin B6, cobalamin (vitamin B12) and choline, may also be needed to optimize MTHFR-related methylation capacity in carriers of MTHFR SNPs. Homocysteine is metabolized through three vitamin B–dependent pathways. It can be either (1) remethylated and recycled as methionine, a reaction catalyzed by either the enzyme betaine homocysteine methyltransferase (in which the co-factor betaine is derived from choline) or (2) by the vitamin B12-dependent enzyme methionine synthase, or (3) homocysteine can be removed from the remethylation cycle by undergoing irreversible B6-dependent transsulfuration via the enzyme cystathionine beta synthase to form cysteine.[26] [27]

Conclusion

The folate, methylation and transsulfuration pathways are not separate entities but comprise an interconnected cellular network whose disruption, at any point, may have far-reaching deleterious effects, one of which is increased free radical production coupled with disruption of glutathione (GSH) production—a surefire recipe for accelerated mitochondrial decay and aging.

Ensuring adequacy of all the nutrient co-factors necessary to restore KM in all these pathways, as well as for mitochondrial oxidative phosphorylation (discussed in Part I of this review), is first line therapy for promoting healthy aging. However, a protocol whose end goal is restoration of KM, while certainly helpful in delaying mitochondrial decay, does not address a more fundamental issue – *why* does human physiology shift from a homeostasic state that repairs and balances itself to one that allows decay to accumulate? Researcher Wulf Dröge has called this shift “the first cause of death,” and his insights into its likely causes may provide the means to opt out of the vicious cycle responsible for the age-associated move from a state of youthful homeostatic repair to a one that promotes mitochondrial decay. This will be the topic of Part III of this review: *Reversing the Age-related Metabolic Shift towards Mitochondrial Decay*.

Read Part I: Delaying the Mitochondrial Decay of Aging

Read Part III: Restoring Mitophagy – the Key To Mitochondrial Rejuvenation