Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atheroscleropathy: The pleiotropic effects of folate supplementation

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Abstract

Homocysteine has emerged as a novel independent marker of risk for the development of cardiovascular disease over the past three decades. Additionally, there is a graded mortality risk associated with an elevated fasting plasma total homocysteine (tHcy). Metabolic syndrome (MS) and type 2 diabetes mellitus (T2DM) are now considered to be a strong coronary heart disease (CHD) risk enhancer and a CHD risk equivalent respectively. Hyperhomocysteinemia (HHcy) in patients with MS and T2DM would be expected to share a similar prevalence to the general population of five to seven percent and of even greater importance is: Declining glomerular filtration and overt diabetic nephropathy is a major determinant of tHcy elevation in MS and T2DM.

There are multiple metabolic toxicities resulting in an excess of reactive oxygen species associated with MS, T2DM, and the accelerated atherosclerosis (atheroscleropathy). HHcy is associated with an increased risk of cardiovascular disease, and its individual role and how it interacts with the other multiple toxicities are presented.

The water-soluble B vitamins (especially folate and cobalamin-vitamin B12) have been shown to lower HHcy. The absence of the cystathionine beta synthase enzyme in human vascular cells contributes to the importance of a dual role of folic acid in lowering tHcy through remethylation, as well as, its action of being an electron and hydrogen donor to the essential cofactor tetrahydrobiopterin. This folate shuttle facilitates the important recoupling of the uncoupled endothelial nitric oxide synthase enzyme reaction and may restore the synthesis of the omnipotent endothelial nitric oxide to the vasculature.

Introduction and background

Homocysteine (Hcy) is a nonessential sulfur-containing amino acid and an intermediary metabolic product derived from the demethylated essential amino acid methionine (figure 1).
Since its discovery in 1932 by the 1955 Nobel Prize recipient Vincent DuVigneaud [1] and its association with premature arteriosclerotic (fibrotic) vascular disease described by Kilmer McCully in 1969 [2], Hcy has emerged as a novel marker of risk for cardiovascular disease.

In the past decade Hcy has become widely accepted as a novel risk marker associated with atherosclerotic cardiovascular disease (CVD) in the coronary, cerebral, and peripheral vascular beds. Additionally, it has been determined that hyperhomocysteinemia (HHcy) is an independent and a graded risk factor for the development of CVD [3-13]. The current role of Hcy as a causative factor still remains controversial and needs to be more fully elucidated.

The important role of oxidative – redox stress and HHcy is biologically plausible because Hcy promotes oxidant injury to vascular cells (particularly the endothelium and the eNOS enzyme reaction) through the auto-oxidation of Hcy, formation of Hcy mixed disulfides, interaction of Hcy thiolactones, and protein homocysteinylation [14-16].

Oxidation of two Hcy molecules yields the oxidized disulfide (homocystine), two protons (H+), and two electrons (e-), while promoting the formation of reactive oxygen species (ROS).

**Figure 1**

**Homocysteine metabolism: methionine – folate cycle and the folate shuttle** Hcy is an intermediate metabolic product of methionine metabolism. Once methionine is demethylated, producing Hcy it can be further catabolized to cystathionine and cysteine via the transsulfuration pathway. This transsulfuration pathway is dependent on the cystathionine beta synthase enzyme (CBS) and vitamin B6. Human vascular cells lack the CBS enzyme and therefore the transsulfuration pathway is not present. In the remethylation cycle termed the Methionine – Folate Cycle, folic acid (folate) serves as the methyl donor to convert Hcy to Methionine. This reaction is dependent on the Methionine Synthase (MS) enzyme and the cofactor vitamin B12. Folate serves not only as a methyl donor but also as hydrogen and an electron donor. This hydrogen and electron donation capability renders folate as a dual source for stabilization of the tetrahydrobiopterin (BH4) Cofactor of the endothelial nitric oxide synthase (eNOS) reaction producing the quintessential endothelial nitric oxide (eNO) and the additional function of being a local endothelial microenvironment antioxidant. These findings have led to the hypothesis of a Folate Shuttle phenomenon. As can be seen there may exist a relative endogenous endothelial folate deficiency resulting in a toxic effect of Hcy accumulation within endothelial cells as a result of this Folate Shuttle.
Also, formation of mixed disulfides contributes to the additional formation of ROS

\[ \text{Hcy-SH + R-SH} \rightarrow \text{Hcy-S-SR + H2O} \rightarrow \text{ROS} \]

\[ \text{Hcy-SH + R1-S-S-R2} \rightarrow \text{R1-S-S-Hcy + R2-SH} \rightarrow \text{ROS} \]

R = any organic compound in the plasma with a thiol group (-SH) accessible to react with Hcy, such as proteins, cysteine, glutathionine, gamma-glutamylcysteine, or cystinylglycine.

Additionally, Hcy may undergo complicated rearrangements to form Hcy thiolactone (a cyclic thioester), which is chemically reactive and acylates free amino groups such as the side-chain lysine groups in proteins. In the process of forming homocysteinylated proteins further oxidative stress develops and homocysteinylated proteins become damaged and may lose their biological activity. This results in the modification of proteins and in particular the modification of low-density lipoproteins (LDL-cholesterol), which contribute to their retention within the intima and subsequent inflammatory foam cell formation associated with atherogenesis.

Aminoacyl-tRNA Synthetase

Bound Hcy adeny late (Hcy~AMP)

Jakubowski has recently been able to demonstrate that Hcy is now considered to be a protein amino acid in humans. In areas of turbulent blood flow the formation of Hcy-N-protein mediated by S-nitroso-Hcy may account for the observation that atherosclerosis originates mostly at branch points and flow dividers in arteries [17].

Recent controversial findings have been published in regards to post percutaneous coronary angioplasty (PTCA) restenosis, one trial has shown a decrease in restenosis and major adverse events of death, nonfatal myocardial infarction, and need for repeat revascularization [18-20], while others have shown no significant effect on the occurrence of restenosis [21-23].

A recent secondary prevention trial by Liem et al. [24] has shown no effect in cardiovascular risk reduction within 2 and now 4 years treatment with low dose folic acid supplementation in patients previously on statin therapy and stable coronary artery disease.

Regardless of the current controversies regarding Hcy as being an independent risk factor or merely a risk marker, Hcy has definitely emerged as one of the novel-multifactorial substrates, which interacts with conventional CVD risk factors of the atherosclerotic process [25,26].

Numerous ongoing clinical trials have yet to determine if lowering Hcy by folic acid supplementation decrease the risk of cardiovascular events or cardiovascular mortality and these trials will be forthcoming within the next few years (table 1).

An attempt to shed some new light on the Hcy – HHcy story as it pertains to the metabolic syndrome (MS), pre-diabetes (PD), and overt type 2 diabetes mellitus (T2DM) and their association with the multiple metabolic toxicities resulting in an increase of reactive oxygen species (ROS) will be discussed.

Furthermore, an attempt to develop a hypothesis that HHcy is a marker of oxidative and redox stress, as a result of an endogenous folate shuttle, which results in a relative endogenous endothelial folate deficiency due to a folate shuttle phenomenon to preferentially run the endothelial nitric oxide synthase (eNOS) reaction will be explored.

The lack of Hcy catabolic transsulfuration in human vascular cells provides an even more important role for the folate shuttle phenomenon in vascular cells. This would result in a relative endogenous endothelial folate deficiency to operate the remethylation pathway via the methionine – folate cycle culminating in vascular cell HHcy.

This folate shuttle phenomenon could also be operative in hypertension and non-diabetic atherosclerosis due to an associated uncoupling of the eNOS enzyme reaction with decreased endothelial nitric oxide (eNO) production and resultant endothelial cell dysfunction.

There are multiple clinical factors associated with HHcy (table 2), as well as, multiple damaging effects to the vascular system (table 3). In MS, PD, and T2DM there are multiple injurious stimuli to the endothelial cell (including HHcy), and these substrate interactions result in accelerated atherosclerosis → atheroscleropathy (figure 2).

While the liver normally contains the full complement of metabolic enzymes to handle HHcy through the remeth-
ylation and the transsulfuration catabolic metabolic pathway, it is limited in human vascular cells. Vascular cells in particular do not express cystathionine beta synthase (CBS), the first enzyme of the hepatic transsulfuration pathway; nor do they express the enzyme betaine homocysteine methyltransferase (BHMT), which catalyzes the alternate remethylation pathway in the liver using betaine as a substrate [27,28].

Therefore in vascular cells, Hcy metabolism is limited to the B12 – folate dependent remethylation pathway catalyzed by methionine synthase of the methionine-folate cycle. Thus, vascular cells and particularly endothelial cells may be especially vulnerable to the higher levels of circulating and endogenous Hcy found in patients with HHcy.

This makes the endothelial cell and smooth muscle cells quite vulnerable to the previously discussed oxidative stress of HHcy. In the MS, PD, and overt T2DM this would definitely result in an additive – synergistic effect to the associated multiple metabolic toxicities and the elevated substrates of the A-FLIGHT acronym (table 4).

These findings would help to explain the increased mortality and cardiovascular disease associated with HHcy, especially in T2DM [29-31]. In concluding this section it is important to note that:

HHcy is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus [8].

**Reactive oxygen species: “toxic oxygen”**

ROS consist primarily of the oxygen free radicals (superoxide $[O_2]^*$), hydroxyl radical $[-\cdot OH]^*$, peroxynitrite $[ONOO]^*$), the potent oxidizing agents of the non-radical family (peroxide $[H_2O_2]$ and hypochlorous acid $[HClO]$), and the organic analogues, which include reactive nitrogen species: specifically, peroxynitrite $[ONOO]^*$ (table 5).

It has been known for some time that ROS are detrimental and toxic to cells and tissues as a result of injury to lipids, nucleic acids, and proteins: (A). Lipid peroxidation of membranes (loss of membrane function and increased permeability) and generation of lipid autoperoxidation reactions. (B). DNA damage leading to mutation and death. (C). Cross linking or vulcanization of sulphhydryl rich proteins (leading to stiff aged proteins specifically collagen of the extracellular matrix) [32].

Both intracellular and extracellular antioxidants play important roles in neutralizing these toxic oxygen molecules. The endogenous antioxidant enzyme family consists primarily of superoxide dismutase (SOD), catalase, glutathione peroxidase and the tri-peptide (gamma-L-glutamyl-L-cysteintyl-glycine) glutathione or (GSH), and free circulating thiols containing the sulfhydryl group (-SH) (table 6). These antioxidants are found to be systemically depleted in the clinical setting of T2DM and evidence is present for their depletion in atheromatous lesions [31,32].

There are multiple metabolic toxicities associated with MS, PD, T2DM, and their companion: accelerated atherosclerosis (atheroscleropathy). These multiple metabolic toxicities can be conveniently grouped in an acronym termed the A-FLIGHT toxicities (table 4). Each of these toxicity substrates result in the excessive production of ROS responsible for the damaging effects of oxidative – redox stress.

Not only are ROS involved in the development of autoimmune type 1 diabetes mellitus and T2DM but also play an important role in the long-term development of the associated complications: atheroscleropathy, diabetic cardiomyopathy, intimalopathy, nephropathy, neuropathy, endotheliopathy, and retinopathy.

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**Table 1: Homocysteine lowering intervention trials for the prevention of CVD.**

| Trial       | Description                                                                 |
|-------------|----------------------------------------------------------------------------|
| **SEARCH**  | Study of the effectiveness of additional reduction in cholesterol and homocysteine. University of Oxford, England. |
| **PACIFIC** | Prevention with a combined inhibitor of folate in coronary heart disease. University of Sydney, Australia. |
| **WACS**    | Women's antioxidant and cardiovascular disease study. Harvard Medical School, USA. |
| **CHAOS-2** | Cambridge heart antioxidant study. University of Cambridge, England. |
| **NORVITE** | Norwegian study of homocysteine lowering with B-vitamins in myocardial infarction. University of Tromso, Norway. |
| **BERGEN**  | Bergen vitamin study. University of Bergen, Norway. |
| **HOPE-2**  | Health outcome and prevention evaluation number 2. Canada. |
| **VISP**    | Vitamin intervention for stroke prevention. Wake Forest University, USA. |
| **VITATOPS**| Vitamins to prevent strokes. Perth, Australia. |
| **IST-2**   | International strokes trial number 2. Auckland, New Zealand. |
Hyperhomocysteinemia and reactive oxygen species in insulin resistance, metabolic syndrome, type 2 diabetes mellitus, and atheroscleropathy

There are multiple metabolic toxicities associated with MS, PD, and overt T2DM, which are associated with the production of ROS. These toxic substrates and their associated ROS have been conveniently grouped together to form the acronym: A-FLIGHT toxicities (table 4).

The general population of patients with MS, IR, PD, and overt T2DM will, in allprobability, have a similar frequency of the common polymorphism affecting the folate-dependent, thermolabile gene coding for the 5, 10-methylene tetrahydrofolate reductase MTHFR (C677T, Ala → Val) is associated with a decreased activity of the enzyme with subsequent mild to moderate HHcy, which occurs in 10–15% of the general population [33-35].

Figure 2

Multiple injurious stimuli to the endothelium, intima, media, and adventitia

The endothelial cell is exposed to multiple injurious stimuli consisting of: modified LDL-cholesterol, various infection insults (viral and bacterial), angiotensin II, hemodynamic stress, LPa, glucose, homocysteine, and intimal redox stress or reactive oxygen species. As discussed in this review, the toxicity of homocysteine may act alone as well as in concert with the other multiple injurious stimuli to injure the endothelium resulting in endothelial cell dysfunction. Especially in the MS, PD, overt T2DM, and atheroscleropathy. It is of importance to note that native LDL-cholesterol is not atherogenic to the vascular intima. The process of oxidation, glycation, glycoxidation, or homocysteinylation must modify LDL-cholesterol in order to become atherogenic. Thus, the importance of the multiple injurious stimuli acting alone and synergistically to modify LDL-cholesterol and accelerate angiogenesis as seen in the accelerated atherosclerosis associated with MS, PD, and overt T2DM termed atheroscleropathy.
This gene polymorphism is especially important in those individuals with a decrease in dietary folate. In these patients mild to moderate HHcy can be improved with dietary and folate supplementation, which improves endothelial-dependent endothelial cell dysfunction [36]. In the general population, mild to moderate HHcy occurs in approximately 5–7% [9].

HHcy is not usually present as a direct result of MS, PD, and T2DM unless there is an associated development of impaired renal function. As nephropathy develops, there is an associated elevation of total Hcy associated with a decline in glomerular filtration rate [37].

In the early latent Stage I and transitional Stage II of T2DM (table 7) homocysteine may actually be lower than expected. Rosolova H et al. have found an unexpected inverse relationship between IR and serum Hcy in a population of healthy subjects with insulin resistance. They have attributed this finding to the associated increase in the glomerular hyperfiltration [38].

IR and PD (Stages I, II, and early Stage III) are associated for a period of time with hyperinsulinemia, hyperproinsulinemia, and hyperamylinemia and glomerular hyperfiltration and hyperperfusion. Recently, the nitric oxide system has been shown to be involved in glomerular hyperfiltration in Japanese normo- and micro-albuminu-
Table 4: MULTIPLE METABOLIC TOXICITIES IN MS AND T2DM: THE A-FLIGHT ACRONYM

| Initiator                      | Metabolic Defect                      | Metabolic mediator | Functional mediator | Consequence                                      |
|--------------------------------|---------------------------------------|--------------------|---------------------|--------------------------------------------------|
| A Amylin (Co-secreted – Co-    | Hyperamylinemia                       | Activation of ANG II | PKC Signal Transduction | ROS IAPP Amyloid in islets contributing to Beta Cell defect. Possible deposition in the intima, mesangium, neuronal unit, and myocardial. REMODELING |
| packaged within the insulin    |                                       |                     |                     |                                                  |
| secretory granule) by the islet |                                       |                     |                     |                                                  |
| Beta cell. Insulin’s ‘fraternal twins’ (Elevated in MS, PD, and Early T2DM) |
| AGE Advanced Glycation         | Ang II Excess                         | Ang II Excess Most potent stimulus for: Activation of Vascular membrane bound Lipotoxicity Lipid Triad FFA ALE | PKC Signal Transduction, Superoxide production, Uncoupling of the eNOS reaction, TGF-beta-1 activation | ROS Myocardial, Renal, Intimal, Retinal, and Neuronal remodeling |
| Endproducts AFE Advanced       |                                       |                     |                     |                                                  |
| fructosylation endproducts     |                                       |                     |                     |                                                  |
| Advanced Lipoxidation Endproducts (ALE) | Protein Cross – linking / Dysfunction for AGE | Matris Defects Signal Transduction Matrix Defects | ROS REDOX STRESS |
| Antioxidant Enzymes:           | Reduced – Dysfunctional                | Decreased NO       | Decreased NO REDOX STRESS |                                                      |
| Antioxidant Enzymes: Absence   |                                       |                     |                     |                                                  |
| of antioxidant network         |                                       |                     |                     |                                                  |
| AGE Advanced Glycation Endproducts AFE See Glucotoxicity (G) RAGE activation Receptor for AGE | Protein Cross – linking | Matris Defects Signal Transduction | ROS REDOX STRESS |
| Increased accumulation of      | Increased Ox-LDL-C, TNFalpha, Capase 3, | Decreased NO: Decreased NO REDOX STRESS | ROS Decreas NO |                                                      |
| multiple metabolic toxicities  | Glomerulosclerosis.                   |                     |                     |                                                  |
| Atherosclerotic Nephropathy    | ROS beget ROS Atheroscleropsy          | Decreased NO Self perpetuating Decreased NO | Decreased NO No Athero – emboli Activated Platelets See Thrombocic Tox. | ROS beget ROS Decreased NO |
| F Free fatty acid toxicity     | Elevated FFA                          | LC acyl-CoA’s Fat Accumulation | Mitochondrial Defects Non Adipose Accumulation of Fat (LC acyl-CoA’s) in Adipose and Non Adipose Tissue | ROS Cytotoxicity |
| L Lipotoxicity Lipid Triad FFA | Increased VLDL – VLDL Triglycerides and Small dense atherogenic LDL-Cholesterol with Decreased HDL-Cholesterol LIPID TRIAD |                      |                     |                                                  |
| ALE Long chain acyl-CA  |                                       |                     |                     |                                                  |
| I Insulin toxicity ENDOGENOUS  | Hyperinsulinemia                       | Ang II Increase # AT-I receptors Cross-talk with AT-1 Increase FFA Increase PAI-I Increase Sympathetic tone and activity Increased Na+ and H2O reabsorption Increase Volume and Blood Pressure Hypertension Hypothesis | REDOX STRESS SIGNAL PATHWAYS | ROS ROS ROS Extracellular Matrix Remodeling Islet, intimal, renal, myocardial, and neuronal. |
| Insulin Resistance             | Hyperamylinemia in , MS, PD, EARLY T2DM Glut 4 is NO dependent Redox sensitive pathway |                      |                     |                                                  |
| Inflammation toxicity, “Inflammatory Cycle” (figure 5) | Activation of the innate immune system: IL-6, IL-1B, TNF alpha Macrophage (MPO) – Hypochlorous Acid Superoxide O2 | Acute Phase Reactions: C-Reactive Protein Serum Amyloid A Fibrinogen | NF kappa B Cellular Adhesion Molecules: ICAM, VCAM, and MCP-1 | ROS Infammation begets Inflammation ” INFLAMMATORY CYCLE “ (figure 3) ROS beget ROS |
| Insulin deficiency             | OVERT T2DM                            | GLUCOTOXICITY POLYOL SORBITOL PATHWAY | REDUCTIVE STRESS | ROS |
| Pent 
| G Glucotoxicity                | Glycation / AGE                       | See above NO quenching Macrophage Activation | See above | See above Dysfunctional Sign Transduction | Ross Infammation begets Inflammation ” INFLAMMATORY CYCLE “ (figure 3) ROS beget ROS |
| Glucotoxicity                  |                                       | Protein inactivation | Vasoconstriction Increased Cytokines, TGF-Beta |                              |
| ORIGIN OF REDUCTIVE STRESS !   | Auto-oxidation                         | Free Radical Formation | REDOX STRESS | CYTOTOXICITY ROS |
| REDUCTIVE STRESS !             | Polyal Sorbitol Pathway (eNO inhibits Aldose Reductase) | Increased NADH Lactate Reductive Stress | REDOX STRESS | CYTOTOXICITY ROS |
| Increased DAG Glucotoxicity    | Increased DAG | Increased PKC | REDOX STRESS | CYTOTOXICITY ROS |
| H Hypertension Toxicity        | RAAS activation HHcy NO quenching and NEW: PAR interaction. | Ang II Decreased Gpx, DDAM with resultant ADMA | NAD(P)H REDOX STRESS | ROS Decreased NO, Endothelial Cell toxicity, dysfunction, and apoptosis |
| Homocysteine Toxicity          |                                       |                     |                     |                                                  |
| T Triglyceride Toxicity Thrombotic | Triglyceride – FFA exchange | See FFA – Lipotoxicity above eNOS uncoupling | REDOX STRESS Activated Platelets PA1 – eNOS uncoupling | ROS Athero-embol ROS |
| Triglyceride toxicity          |                                       |                     |                     |                                                  |
| Triglyceride (antioxidant)     |                                       |                     |                     |                                                  |
| depletion                      |                                       |                     |                     |                                                  |
ric patients with T2DM and early stages of diabetic nephropathy [39]. Hiragushi K et al. were able to demonstrate an increase staining of eNOS enzyme (in glomerular endothelial cells) in microalbuminuric T2DM patients in comparison to non-diabetic control subjects.

This adaptive early response results in glomerular enlargement and an increase in the glomerular filtration rate [40]. Additionally, Apakkan Aksun S et al. found that serum and urine nitric oxide levels were elevated, as well as, plasma malondialdehyde (MDA), which points to the importance of oxidative stress in patients with T2DM. These authors feel that the high nitric oxide levels may lead to hyperfiltration and hyperperfusion, which in turn leads to an increase in urinary albumin excretion and thus causes progression of nephropathy in early T2DM [41].

Over time (Stages III-V, table 7) there is an increase in glucotoxicity (initially postprandial Stage III and fasting in Stage IV), as well as, an increase in redox and oxidative stress to the glomerular endothelium. This damage is associated with lipid peroxides, free fatty acids, and activation of Ang II as a result of hyperinsulinemia, hyperproinsulinemia, hyperamylinemia and the totality of reactive oxygen species associated with the A-FLIGHT toxicities.

The activation of Ang II would be associated with the activation of the membranous NAD(P)H oxidase enzyme and generation of superoxide with its effect on endothelial cell dysfunction and uncoupling of the eNOS reaction. Over time, this would result in decreases in eNO and loss of a natural occurring local antioxidant network within the glomerular endothelial microenvironment.
Glucotoxicity is associated with advanced glycosylation endproducts (AGEs) and their receptor (RAGE), activation of aldose reductase of the polyol-sorbitol pathway, activation of protein kinase C (PKC) isoforms, and transforming growth factor beta (TGFβ). Once these glucotoxic mechanisms are in play and overt diabetic nephropathy with associated glomerulosclerosis and remodeling has developed, there will be a progressive decrease in glomerular filtration. These changes will result in Hcy elevation [37].

Thus, HHcy plays an extremely important role for additional oxidative-redox stress regarding the accelerated cardiovascular disease observed in diabetic patients on dialysis [42].

HHcy is thought to induce an oxidative inactivation of endothelial nitric oxide, in part by inhibiting or consuming the expression of cellular glutathione peroxidase (GPx). In heterozygous cystathionine beta-synthase deficient +/- mice, Weiss et al. were able to restore endothelial cell function by increasing cellular thiols and reduced glutathione pools and increasing GPx activity with restoration of the endothelial function [43].

The ensuing cellular redox stress is magnified and total Hcy consumes NO by the indirect process of superoxide (O$_2^-$) converting NO to toxic peroxynitrite (ONOO$^-\$).

In addition to ONOO$^-\$ formation, NO in conjunction with thiols and oxygen radicals generate nitrotyrosine and nitroarginine. Nitroarginine, in turn, competes for the substrate L-arginine of the eNOS reaction in a feedback mechanism, limiting further NO generation [44-46]. As a result, there is endothelial cell dysfunction, endothelial cell toxicity and endothelial cell loss-apoptosis, increased ROS, increased ONOO$^-\$ and decreased NO associated with HHcy [47]. HHcy is multiplicative in nature and even though its effects may occur later in T2DM than the other associated A-FLIGHT substrate toxicities, it has a devastating – synergistic effect on endothelial cell function. Presently, we know there are other multiple metabolic toxicities (table 4) operating within the renal glomerulus producing microalbuminuria (reflecting endothelial cell

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**Table 7: The Five Stages of T2DM:**

| Stage                  | Description                                                                 |
|------------------------|-----------------------------------------------------------------------------|
| **I. LATENT STAGE**:   | **EARLY** Insulin Resistance: Genomic component. Modifiable: obesity/sedentary life style. Nonmodifiable: aging |
| **BETA CELL DEFECT**:  | *(Dysfunction)* Abnormal processing, storage or secretion.                 |
| **ISLET AMYLOID**:     | Diffusion Barrier: Secretory Defect: Intra Islet Absorptive Defect:         |
| **II. TRANSITION STAGE**: | Persistent Hyperinsulinemia, Hyperproinsulinemia. → Ang II Accelerated Atherosclerosis |
| **III. IGT STAGE**:    | *(Impaired Glucose Tolerance)*: Pre-diabetes Human Health Services (HHS) and American Diabetes Association (ADA) term. |
| **IV. IFG STAGE**:     | *(Impaired Fasting Glucose)*: Pre-diabetes Human Health Services (HHS) and American Diabetes Association (ADA) term. |
| **V. OVERT STAGE**:    | **TOO LATE** FBG 126 or greater: Random or 2 hour OGTT 200 or > |

**Paradigm Shift.** → Diagnose Early → Start treatment Early → Stage III: IGT.
dysfunction and damage) at a stage prior to the declining glomerular filtration rate responsible for HHcy (figure 2).

A recent clinical study by Maejima et al. [48] revealed significant elevated levels of ONOO− peroxynitrite (by Griess method) in 129 T2DM patients as compared to 76 non-diabetic controls. While there was no difference in basal plasma NO(2)(−) levels between the T2DM and control patients, ONOO− levels were related to the presence of hypertension and advanced microvascular complications. In addition, ONOO− correlated positively with elevations in AGEs and serum lipid peroxide. These data support the hypothesis that decreased endothelium-dependent vasodilatation in diabetic subjects is associated with the impaired action of NO secondary to its consumption (quenching) from redox stress rather than decreased NO production from vascular endothelium. Clinically, abnormal NO metabolism is related to advanced diabetic microvascular complications.

Zhang et al. [49] were able to demonstrate that increased concentrations of Hcy resulted in a decreased NO response to bradykinin and L-arginine. Hcy stimulated the formation of superoxide anions and peroxynitrite with increased levels of nitrotyrosine. The addition of 5-methyltetrahydrofolate (folic acid) restored NO responses to bradykinin and L-arginine agonists. In addition, scavengers of peroxynitrite and SOD mimetics reversed the Hcy-induced suppression of NO production by endothelial cells. Concentrations of Hcy (greater than 20 micromolar concentration) produced a significant indirect suppression of eNOS activity without any discernible effects on its expression.

Li et al. just published an article showing an unexpected effect of Hcy-induced oxidative stress resulting in an increase of 3-hydroxy-3-methylglutaryl coenzyme A reductase in vascular endothelial cells, as well as decreasing endothelial NO. They were also able to demonstrate that “statins” increased NO as well as decreasing cellular cholesterol [50].

Stuhlinger et al were able to demonstrate that Hcy impairs the nitric oxide synthase pathway. Homocysteine inhibits dimethylarginine dimethylaminohydrolase (DDAH), which is responsible for degrading ADMA. This effect of Hcy causes the endogenous inhibitor of nitric oxide synthase, ADMA, to accumulate and compete with L-arginine for nitric oxide production. This effect helps to explain the deleterious effect of Hcy on the endothelial cells ability to promote vasodilatation and associated endothelial cell dysfunction with decreased NO synthesis [51].

Previous publications have emphasized the importance of the eNOS enzyme and the important role of the Glu298→ Asp gene polymorphism [32,52]. Noiri and colleagues have recently demonstrated that the gene polymorphism (Glu298→ Asp) affecting the eNOS enzyme is statistically and significantly associated with diabetic nephropathy in T2DM patients [53]. This finding points to the important role of the eNOS enzyme and eNO in controlling oxidative – redox stress and its relation to the development of diabetic nephropathy.

This same gene polymorphism (Glu298→ Asp) of the eNOS enzyme (eNOS 894TT genotype) has also been recently demonstrated to be a risk factor for HHcy. These findings by Brown et al. indicate that the NOS3 894TT genotype (eNOS Glu298→ Asp gene polymorphism) is a risk factor for HHcy in healthy nonsmoking adults with low serum folate and supports the hypothesis that nitric oxide modulates Hcy through an effect on folate catabolism [54].

**Accelerated atherosclerosis → atheroscleropathy**

The above discussion also applies to the accelerated atherosclerosis (atheroscleropathy) and intimal redox stress and remodeling (intimopathy) associated with MS, PD, and overt T2DM [32].

Insulin resistance is central to the development of CHD and the accelerated atheroscleropathy associated with MS, PD, and overt T2DM. This being said, the ultimate manifestation of insulin resistance is T2DM [32] (figure 3).

Atherosclerosis, MS, PD, and T2DM present a pleiotropic mechanistic progression, in which, inflammation and upstream oxidative-redox stress and ROS appear to share a common – fertile soil background, and patients with diabetes experience both a preexisting and parallel-accelerated atherosclerosis, we have termed atheroscleropathy [32,52,55] (figure 4).

In regards to silent coronary artery disease (CAD), Gazzaruso C et al. were able to confirm that microalbuminuria and smoking may predict silent CAD in people with diabetes and additionally they were able to demonstrate an original finding of an independent association between HHcy and silent CAD [56].

In the Framingham offspring study [57], those with hyperinsulinemia had significantly higher Hcy levels than those without, and subjects with more than two MS phenotypes (hypertension, impaired glucose tolerance, or a central MS [consisting of two or more of the following: hyperinsulinemia, obesity, or dyslipidemia]) had significantly higher Hcy levels compared to those with one or no MS phenotype.
Passaro and colleagues have been able to demonstrate in T2DM patients that Hcy levels are influenced by both the duration of the disease and metabolic control. Hcy levels improve with improvement of glycemic control of HbA1c. Hcy remained strongly associated to the presence of CHD independent from age, sex, body mass index, smoking status, hypertension and lipid patterns. Additionally, they conclude that Hcy can be seen as a major risk factor in T2DM patients with CHD [58,59].

The initial vascular lesions described by McCully in 1969 [2] described an arteriosclerotic fibrotic change within the intima, which is in contrast to the intimal fibrous-lipid laden lesions associated with atherosclerosis and the vulnerable thin-cap fibroatheroma prone to rupture.

Burke and colleagues have been the first to describe the plaque morphology and correlate these findings to total plasma homocysteine [60]. Their recent publication demonstrated that Hcy is elevated in sudden unexpected death in men as a result of severe coronary artery disease without acute or organized coronary thrombosis. Additionally, they were able to demonstrate that the above association seemed to be increased if there existed concomitant diabetes mellitus. HHcy was associated with an increase in fibrous plaques and a relative decrease in thin-cap atheromas. Their findings suggest a different mechanism of atherogenesis from that of hypercholesterolemia. Additionally, efforts to reduce Hcy could be complementary to lipid lowering therapy because plaque fibrosis may occur via a distinct mechanism. They suggest that one
such mechanism could be endothelial dysfunction, which certainly points to the importance of the "Folate Shuttle phenomenon" within the endothelial cell and a relative endogenous endothelial folate deficiency within the endothelial microenvironment milieu.

These morphological findings suggest the need for; and importance of; global risk reduction of the entire spectrum of elevated substrates within the A-FLIGHT multiple metabolic toxicities.

**PPAR and homocysteine**

Peroxisome proliferator activated receptors (PPARs) are transcription factors belonging to the nuclear receptor super-family and the multiple roles of their effects are rapidly unfolding.

Clinically there are two classes of drugs currently in use: fibrates binding to PPAR alpha (gemfibrozil and fenofibrate) and thiazolidinediones (TZDs) binding primarily to PPAR gamma (rosiglitazone and pioglitazone). PPAR alpha agonists are responsible primarily for their hypolipidemic effect, while PPAR gamma agonists are antidiabetic agents – insulin sensitizers (enhancing insulin – mediated glucose uptake and lowering of HbA1C), important in the regulation of the lipid metabolism and adipogenesis, and additionally expressed in the vasculature (endothelial cells, smooth muscle cells, and monocyte – macrophage cells). In vitro and clinical data show that TZDs can decrease thrombotic, inflammatory, and oxidative changes that contribute to endothelial dysfunction and their future vaculoprotective roles (table 8) in attenuating the progressive role of non-diabetic atherosclerosis.

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**Figure 4**

The reactive oxygen species – inflammatory cycle: ROS are upstream from the inflammatory cycle

Inflammation in MS, PD, overt T2DM, and atheroscleropathy has recently emerged as an important factor. While it has been interesting to observe the development of this exciting story, it is important to remember that reactive oxygen species (ROS) cycle occurs upstream from the inflammatory cycle via the redox sensitive nuclear transcription factor: NF kappa B and the resulting inflammatory markers: Selectins, cellular adhesion molecules, monocyte chemoattractant protein - 1, and the cytokines: TNF alpha, IL-6, IL-8 and the newer clinical laboratory tool of the highly sensitive C-reactive protein (hsCRP).
and the accelerated atherosclerosis in MS, PD, and overt T2DM [61-67].

Current available information suggests that PPAR-gamma ligands may prevent the progression of insulin resistance (preserving islet Beta cell function) to diabetes and endothelial dysfunction to atherosclerosis [68].

Recently, Fonseca V. et al. were able to demonstrate that the PPAR agonist troglitazone had a significant Hcy lowering effect in both the lean and fatty Zucker rat model of insulin resistance. This decline in Hcy was associated with significant increases in hepatic concentrations of S-adenosylhomocysteine (SAH) and S-adenosylmethionine (SAM) plus SAH. Additionally, there was a significant decline in the SAM/SAH ratio. The hepatic CBS was significantly higher in the troglitazone treated group as compared to the control group. The authors conclude that the Hcy lowering effect of troglitazone may be due to a shift in the concentrations of Hcy from the blood to the liver, as well as, a possible upregulation of hepatic CBS [69].

Tao et al. just published a timely article that ties together quite nicely the detrimental role of oxidative-redox stress and ROS and the antioxidant, antiininitiative, and vasculoprotective role of PPARs. They were able to demonstrate (in hypercholesterolemic rabbits) that treatment with the PPAR gamma agonist rosiglitazone enhanced PPARgamma expression, improved endothelium-dependent vasodilatation, preserved the phosphorylation of vasodilator-stimulated phosphoprotein (P-VASP), suppressed gp91(phox) of the membranous NAD(P)H Oxidase enzyme, reduced superoxide and total NOx (stable metabolic byproducts of NO) production, and inhibited nitrotyrosine formation. This may have definite clinical applications regarding the actions of the PPAR agonist family of fibric acid, as well as the PPAR agonists: TDZs (rosiglitazone and pioglitazone currently in clinical use). If Hcy is elevated there may exist a competitive inhibition of the agonist effects of both of these clinically useful medications (figure 5).

Table 8: POSITIVE EFFECTS OF PPARalpha AND PPARgamma AGONISTS

| PPAR alpha: Lipid Effects |
|---------------------------|
| a). Primarily a VLDL-Cholesterol (triglyceride) by increasing lipoprotein lipase and secondary LDL-Cholesterol lowering. |
| b). HDL-cholesterol raising effect by increasing the expression of apo A-I and apo A-II. Positive outcomes of the Fibric acid (Gemfibrozil) (VA-HIT) and Fenofibrate (DAIS) studies. |

| PPAR gamma effects: |
|---------------------|
| a). Primary Clinical Action: (Insulin Sensitizers). Improving insulin mediated glucose uptake. |
| b). Decrease thrombocytic, inflammatory and oxidative changes that contribute to endothelial cell dysfunction. |
| c). Improve vascular reactivity. |
| d). Decrease plasminogen activator inhibitor-I (PAI-I). |
| e). Decrease monocyte expression NFkappa-B. |
| f). Anti-inflammatory effects: Inhibition of macrophage activation and the production of inflammatory cytokines (TNFalpha, interleukin 6 and 1beta). |
| New findings: May be through the effects of PPARdelta. |
| g). Decrease C-reactive protein and IL-6 (both markers of inflammation and cardiovascular risk). Dandonna P reference. Diabetes Technol Ther. 2002; 4(6): 809–15. |
| h). Inhibit vascular SMC migration and proliferation. |
| i). Lower blood pressure. |
| j). Attenuate myocardial hypertrophy and protect against ischemia-reperfusion injury. |
| k). Preserve pancreatic islet Beta cell function. |
| l). Attenuate (specifically rosiglitazone) oxidative and nitrosative stress: enhanced PPAR gamma expression, improved endothelium – dependent vasodilation, preserved P-VASP, suppressed gp91(phox) of the membranous NAD(P)H Oxidase enzyme, reduced superoxide and total NOx (stable metabolic byproducts of NO) production, and inhibited nitrotyrosine formation. |
| m). Increase levels of Adiponectin. |

This may have definite clinical applications regarding the actions of the PPAR agonist family of fibric acid, as well as the PPAR agonists: TDZs (rosiglitazone and pioglitazone currently in clinical use). If Hcy is elevated there may exist a competitive inhibition of the agonist effects of both of these clinically useful medications (figure 5). These findings of the potential interaction of Hcy with the PPAR ligands may interfere with the positive role of glucose -lipid regulation and anti-inflammatory
antiatherosclerotic actions of the fibric acid derivatives and thiazolidinediones.

Homocysteine – folate – cobalamin triad and atherogenesis
Flynn MA et al. have discussed atherogenesis and the Homocysteine-Folate-Cobalamin (HFC) Triad and it seems pertinent to expand each of their respective roles in this discussion [75].

The toxic role of Hcy on the vasculature has been extensively discussed previously, however, the important role of folic acid and cobalamin (vitamin B₁₂) needs further development.

Folic acid and the folate shuttle
Folic acid, the water soluble B vitamin (Vitamin B₉), 5-methyltetrahydrofolate (5-MTHF), has been recently gaining considerable attention due to its positive role in cardiovascular disease, neurodegenerative disorders, neural tube defects, and cancer. Folic acid plays an important role in the remethylation (methionine – folate cycle) of homocysteine and is thus capable of lowering elevated levels of homocysteine [76].

Deficiency or impairment of folate metabolism is associated with HHcy, hypomethylation (the decreased one carbon unit transfer to purines and pyrimidines for DNA repair and biosynthesis), DNA damage, and impaired cell proliferation, malignancies, and impaired eNO production [47,77-80].
5-MTHF is an electron donor, a hydrogen donor, and a methyl donor [52] and this attribute confers a unique role to its function. In regards to the eNOS reaction, it supplies both hydrogen and electrons to the oxidatively modified tetrahydrobiopterin (BH4): BH2 and BH3. Once biopterin is completely reduced to BH4 (the necessary cofactor for the eNOS reaction), the eNOS reaction can re-couple and restore the capability of this reaction to be once again a net producer of eNO instead of superoxide. This unique function of folic acid can restore endothelial dependent vasodilation in T2DM and hyperlipidemia [81].

Folic acid supplementation would supply the methyl donating substrate to run the methionine-folate cycle, as well as, supply the hydrogen and electrons to stabilize the requisite BH4 cofactor to run the eNOS reaction resulting in the production of eNO by the endothelium. The restoration of the eNOS reaction, by folate supplementation, has been demonstrated acutely by flow mediated dilation studies in T2DM and coronary artery disease via mechanisms, which are largely independent of Hcy lowering [82,83].

We hypothesize that there may exist a folate shuttle, which is operative in the atheroscleropathy associated with MS, PD, and T2DM, as well as, non-diabetic atherosclerosis. This folate shuttle effect may operate as an effective mechanism in the endothelial microenvironment to recouple the oxidatively stressed, uncoupled, and dysfunctional eNOS reaction (figures 1).

There are three critical arms associated with the eNOS reaction: the eNOS enzyme, the L-arginine substrate, and the BH4 cofactor. Each of these three arms is crucial and has been discussed in previous publications [31,32,52,84]. The eNOS reaction may be uncoupled due to oxidative-redox stress associated with MS, PD, T2DM, atheroscleropathy, and non-diabetic atherosclerosis. When the eNOS reaction is uncoupled the reaction will become a net producer of superoxide (instead of eNO via the eNOS reaction) through the reaction of membranous NAD(P)H, oxygen, and the NAD(P)H Oxidase enzyme (figure 6).

The following eNOS reaction demonstrates the location of the vulnerable three arms of the eNOS reaction responsible for the generation of eNO (figure 6).

\[
\text{L-arginine} \xrightarrow{\text{(1)}} \text{NAD(P)H} \xrightarrow{\text{(2)}} \text{NAD(P)H Oxidase} \xrightarrow{\text{(3)}} \text{BH4} \xrightarrow{\text{NO + L-citrulline + NAD (P)}} + \text{O}_2'
\]

If the eNOS reaction is uncoupled: there is the net production of superoxide instead of eNO.

The healthy endothelium is a net producer of endothelial nitric oxide [eNO].

The activated – dysfunctional – uncoupled endothelium is a net producer of superoxide [O$_2^+$] associated with MS, PD, T2DM, and atheroscleropathy.

The net production of superoxide by the uncoupled eNOS reaction in addition to the production of ROS via the A-FLIGHT metabolic toxicities results in an excessive oxidative-redox stress.

This oxidant stress may contribute to the oxidation of BH4 to BH2 and BH3, which will be unable to run the eNOS reaction. Folate supplementation as a methyl donor, a hydrogen donor, and an electron donor may restore the oxidized BH2 and BH3 to the requisite completely reduced BH4 by donating hydrogen and an electron to BH2 and BH3. With the restoration of BH4, the eNOS reaction may recouple and once again run the eNOS reaction in order be a net producer of eNO.

As folate is being shared, stolen, shunted, or shuttled from the remethylation methionine-folate cycle to the eNOS reaction there will develop a relative endogenous endothelial folate deficiency with ensuing intra endothelial Hcy accumulation.

The HHcy that results will then play its own additive auto-oxidative role in the continuing viscous cycle of oxidative stress to the endothelial microenvironment discussed previously.

If this endothelial folate shuttle does exist, then one might assume that Hcy could be a marker of underlying endothelial oxidative – redox stress in addition to being a risk factor. The importance of HHcy being a dual risk (a risk marker and a risk factor) in this patient population certainly points to its importance in being both a result of oxidative – redox stress and its important causative role in endothelial dysfunction.

The importance of the endothelial cell lacking the CBS enzyme only contributes to the hypothesis of an endothelial folate shuttle. Even if measured plasma folate and Hcy levels are normal there may exist a relative endogenous endothelial folate deficiency within the endothelial microenvironment due to the folate shuttle phenomenon.
Cobalamin (vitamin B₁₂)

HHcy is a sensitive marker of clinical cobalamin (the water soluble vitamin B₁₂) deficiency [85,86]. Folate fortification as well as a more widespread use of folate supplements will have an effect in reducing the frequency of folate deficiency and cobalamin deficiency may be ranked above folate deficiency as a primary modifiable cause of HHcy [87-89].

Common causes of cobalamin deficiency are malabsorption as a result of (i). lack or dysfunction of gastric intrinsic factor (ii). hypochlorhydra as a result of chronic gastritis, gastric atrophy, or the use of proton pump inhibitors (iii). the clinical syndromes of inflammatory bowel disease and celiac disease.

It is of interest to note that one of our newer medications in our treatment armamentarium for MS, PD, and T2DM is emerging as a common cause of cobalamin malabsorption. Metformin use has recently skyrocketed as a popular treatment for the metabolic derangements associated with MS, PD, and T2DM. This more novel, yet increasingly, common cause of cobalamin deficiency in adults and teenager necessitates a careful approach for the clinician. Currently, in patients with vitamin B12 deficiency or evidence of peripheral neuropathy or macrocytic anemia the use of metformin should always be considered in the dif-
Folates and cobalamin deficiencies may mask the megaloblastic anemia associated with cobalamin deficiency, while allowing the neurological manifestations of pernicious anemia (peripheral neuropathy, cognitive disturbances, dementia, and depression) to progress. Therefore it imperative that cobalamin deficiency be aggressively sought out and treated, especially in the elderly. In the United States there are 37 million people over the age of 65 with conservative estimates indicating a two to three percent (1.1 million) prevalence either having or will develop pernicious anemia. Additionally, there are estimated to be 20–30 percent (7.4 – 11.1 million) of this population having subclinical cobalamin – B12 deficiency, which may lead to HHcy.

Flynn and colleagues [88] were able to demonstrate in their Longitudinal Aging Study [75] that B12 supplementation (100 microgram by mouth daily) was effective (P value 0.0067) in alleviating HHcy in those patients with a baseline B12 level < 350. In a meta-analysis of Hcy–lowering studies, folic acid by mouth (0.5–5 mg daily) resulted in a reduction of Hcy by 25% (P < 0.001). The addition of 0.5 mg vitamin B12 by mouth daily reduced the Hcy levels by another 3% to 10% over the reduction with folic acid alone. It was interesting to note that the addition of 16.5 mg vitamin B6 daily produced no significant supplemental reducing effect on blood Hcy levels [89,92,93].

**Conclusion**

The multiple metabolic A-FLIGHT toxicities (table 4) observed in MS, PD, T2DM, and atheroscleropathy are associated with an increase in ROS production. These ROS result in an increase in oxidative-redox stress and contribute to the uncoupling of the eNOS reaction and endothelial cell dysfunction.

When accessing the role of the dual risk of HHcy, one has to be aware of the synergistic effects of ROS produced by the other multiple toxicities of the A-FLIGHT acronym and their contributing role of oxidative-redox stress to the endothelium. This points to the important concept of global risk reduction when dealing with MS, PD, T2DM, and atheroscleropathy.

The current controversy of HHcy being a marker of risk or a risk factor (important in causation) is not as important as understanding its overall role in the association with endothelial cell damage, dysfunction, and a decrease in eNO bioavailability and how it interacts with other ROS.

The importance of a possible endothelial folate shuttle points to the important role of folate supplementation in these at risk patients in order to recouple the eNOS reaction and increase eNO production in addition to its remethylation properties in the methionine-folate cycle resulting in lowering of Hcy.

Recently we have become aware of the important role of highly sensitive C reactive protein (hsCRP) and the role of inflammation in the development and progression of cardiovascular disease, MS, PD, and T2DM [94-98]. It has been interesting to watch the hsCRP story unfold and since oxidative – redox stress occurs upstream from the inflammatory cycle via the activation of NFkappa B it seems so very important to better understand the role of HHcy and how it interacts with the ROS produced by the other metabolic toxicities of the A-FLIGHT acronym (figure 4).

Dietary folate supplementation is extremely important for proper endothelial cell health maintenance and function. A simplified overview of the positive roles of folate supplementation include the following [99]:

I. Folate is a methyl donor. Important in lowering the toxic Hcy elevation. Hcy elevation may be a marker of folate deficiency or dysfunction.

II. Folate is a hydrogen donor. Important in maintaining and stabilizing the essential tetrahydrobiopterin (BH4) Cofactor of the eNOS enzyme reaction to prevent uncoupling.

III. Folate is an electron donor. Important as an antioxidant to reduce oxidative – redox stress. Important as a chain breaking antioxidant. Stabilizing the eNOS reaction and protecting the generation of eNO.

Numbers II and III could be thought of as pleiotropic effects: The effects of folate supplementation independent of Hcy lowering, which play a significant role in the stabilization of the eNOS reaction and production of eNO as discussed in the section regarding the FOLATE SHUTTLE phenomenon.

Therefore, in addition to nutritional – dietary folate intake [100], pharmacological folate supplementation should now be strongly considered as part of the clinical global risk reduction treatment paradigm for those at risk patients with MS, PD, T2DM, and atheroscleropathy.
Controversy exists as to who should be screened and who should be treated. The following questions are appropriate:

Should we do not test and do not treat?

Should we treat all patients because it makes good clinical sense and therapy is inexpensive?

Should we screen patients with established atherosclerotic and venous thromboembolic disease and high-risk patients with MS, T2DM, and atheroscleropathy and initiate therapy if Hcy is at or above the 9–15 micromol/L range?

The later is preferred and it may be suggested that the current treatment guidelines for the treatment of HHcy should be one of cardiovascular global risk reduction (table 9) of oxidative – redox stress and reactive oxygen species generated from the multiple metabolic toxicities (table 4). In addition to global risk reduction, current suggested guidelines for the treatment of HHcy as a result of this review are presented in (table 10) [101].

The significance of proper nutrition is of paramount importance; however, once moderate to severe HHcy is identified, existing concepts indicate that diet alone is insufficient to normalize Hcy levels in many patients. This brief review has attempted to provide the reader with a database of knowledge regarding the current role of Hcy and how it interacts with the MS, T2DM, and atheroscleropathy. For those interested in a more in depth review consideration should be given to review the new Journal: METABOLIC SYNDROME AND RELATED DISORDERS. Volume 1 number 2 has been dedicated to homocysteine and the metabolic syndrome [102-110].

Soinio M et al. have just published an exciting article involving a large cohort of patients (462 men and 368 women in the age group of 45–64 years of age at baseline) with T2DM. They were able to demonstrate that plasma Hcy was a strong and independent risk factor for CHD events. Even though impaired renal function is associated with higher plasma Hcy concentrations, this group was able to show that the association between elevated plasma Hcy level and high incidence of CHD events did not depend on renal function [111].

The damaging – toxic role of Hcy in the vascular bed has been well documented in the medical literature and its diffuse role not only in MS, T2DM, and atheroscleropathy but also in multiorgan damage is emerging [80] (figure 7).

To summarize, HHcy in the general population is an independent risk factor for cardiovascular disease as occurs in thrombotic events (such as arterial and venous occlusion) and ischemic disease such as stroke and myocardial infarction (two of the most common causes of death and dis-

Table 9: THE RAAS ACRONYM: GLOBAL RISK REDUCTION

| R | Reductase inhibitors (HMG-CoA). Decreasing modified LDL-cholesterol, i.e. oxidized, acetylated LDL-cholesterol. Decreasing triglycerides and increasing HDL-cholesterol Improving endothelial cell dysfunction. Restoring the abnormal Lipoprotein fractions. Thus, decreasing the redox and oxidative stress to the arterial vessel wall and myocardium. Redox stress reduction. |
|---|---|
| A | AngII inhibition or blockade: ACEi-prils. ARBS-sartans. Both inhibiting the effect of angiotensin-II locally as well as systemically. Affecting hemodynamic stress through their antihypertensive effect as well as the deleterious effects of angiotensin II on cells at the local level – injurious stimuli -decreasing the stimulus for O₂⁻ production. Decreasing the A-FLIGHT toxicities. Plus the direct-indirect antioxidant effect within the arterial vessel wall and capillary. Antioxidant effects. Aspirin antiplatelet, anti-inflammatory effect. Adrenergic (non-selective blockade) in addition to its blockade of Prorenin → Renin Amlodipine with its calcium channel blocking antihypertensive effect, in addition to its direct antioxidant effects. Redox stress reduction. |
| S | Statins. Improving plaque stability (pleiotropic effects) independent of cholesterol lowering. Reducing endothelial cell dysfunction. Statins also decreasing modified LDL cholesterol, i.e. glycated – glycoxidated LDL cholesterol. Improving endothelial cell dysfunction. Also decreasing glucotoxicity and the oxidative – redox stress to the intima and pancreatic islet. Aggressive control of diabetes to HbA1c of less than 7. (This usually requires combination therapy with the use of: Insulin secretagogues, insulin sensitizers (thiazolidinediones), biguanides, alpha-glucosidase inhibitors, and ultimately exogenous insulin.) [36-38] Decreasing modified LDL cholesterol, i.e. glycated – glycoxidated LDL cholesterol. Improving endothelial cell dysfunction. Also decreasing glucotoxicity and the oxidative – redox stress to the intima and pancreatic islet. Aggressive control of blood pressure, which usually requires combination therapy, including thiazide diuretics to attain JNC 7 guidelines. Aggressive control of Hcy with folic acid and its associated pleiotropic positive effect on re-coupling the eNOS reaction by restoring the activity of the BH4 cofactor to run the eNOS reaction and once again produce eNO, as well as, its direct antioxidant effects: BH4 and eNOS stabilization Redox stress reduction. |

| Style: | Lifestyle modification: lose weight, exercise, and change eating habits. Stop Smoking Redox stress reduction |

http://www.nutritionj.com/content/3/1/4
ability). With an aging population who are at greater risk of developing these morbid and mortal cardiovascular diseases associated with MS, T2DM, and atherosclerosis, there should exist a dedicated consideration for folate and possible cobalamin supplementation, in addition to global risk reduction.

Abbreviations

Ang II: angiotensin II; RAAS: renin angiotensin aldosterone system; ROS: reactive oxygen species (O₂*, -OH*, H₂O₂, 1O₂); AT-1: angiotensin type one receptor; PKC: protein kinase C; IAPP: islet amyloid polypeptide; TGF-beta –1: transforming growth factor beta- 1; NAD(P)H oxidase: nicotine adenine di nucleotide phosphate oxidase; AGE: advanced glycation endproducts; AFE: advanced lipoxidation endproducts; eNOS: Endothelial Nitric Oxide Synthase; NO: nitric oxide; BH4: tetra hydro biopterin; FFA: free fatty acids; LC acyl –CoA's: long chain acyl Co enzyme A; VLDL: Very low density lipoprotein; LDL: low density lipoprotein; HDL: high density lipoprotein; MS: Metabolic Syndrome; PD: prediabetes; T2DM: type 2 diabetes mellitus; PAI-1: plasminogen activator inhibitor –1; H₂O: water; Glut-4: glucose transporter-4; PI3 Kinase: phosphatidyl inositol 3 kinase; Akt: protein kinase B; MAP Kinase: mitogen activated protein Kinase; MAP Kinase Shunt: The shunting away from the positive Glut 4 PI3 Kinase Akt pathway to the deleterious MAP Kinase pathway promoting remodeling due to an alteration in the NO redox sensitive PI3 Kinase /Akt pathway; IL-6 IL-8: interleukin-6 interleukin-8; TNF alpha: tumor necrosis factor alpha; MPO: myeloperoxidase: Generation of Superoxide (O₂) via hypochlorous acid HClO –; NF kappa B: nuclear factor kappa B; ICAM: inter cellular adhesion molecule; VCAM: vascular cellular adhesion molecule; MCP-1: monocyte chemoattractant protein-1; NADH: nicotinamide adenine dinucleotide reduced; NAD+: nicotinamide adenine dinucleotide oxidized; DAG: diacylglycerol; GPx: glutathione peroxidase; DDAH: dimethylarginine dimethylaminohydrolase; ADMA: asymmetrical dimethyl arginine; O₂* – ONOO*: superoxide – peroxynitrite.

### Table 10: SUGGESTED TREATMENT GUIDELINES FOR HHcy

| Fasting plasma total Hcy (tHcy) levels: | Relative risk of all cause death | Relative risk of CAD death |
|----------------------------------------|----------------------------------|---------------------------|
| Normal: 5–15 micromol/L (Based on table below 5 – 9 micromol/L) | 1.0 | 1.0 |
| Moderate: 15–30 micromol/L | 1.9 (0.7 – 5.1) | 2.3 (0.7 – 7.7) |
| Intermediate: 31–100 micromol/L | 2.8 (0.9 – 9.0) | 2.5 (0.6 – 10.5) |
| Severe: >100 micromol/L | 4.5 (1.2 – 16.6) | 7.8 (1.7 – 35.1) |
**Figure 7**
The homocysteine wheel: multiorgan damage through redox stress Homocysteine is capable of causing multi-organ damage through the process of oxidative – redox stress. The endothelium is very vulnerable to the effects of HHcy because the endothelium lacks the CBS enzyme, which results in the local loss of the important catabolic metabolism of Hcy. While this article has focused on the cardiovascular manifestations associated with MS, PD, and T2DM there exists numerous other organ systems and diseases associated with the damaging effects of HHcy.

**Competing interests**
None declared.

**Authors’ contributions**
MRH and SCT contributed equally in the inception, writing, and editing of this manuscript.
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