Review

Intimal redox stress: Accelerated atherosclerosis in metabolic syndrome and type 2 diabetes mellitus. Atheroscleropathy
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Keywords: Atherosclerosis, Atheroscleropathy, Oxidative stress, ROS (reactive oxygen species), RNS (reactive nitrogen species), Reductive stress

Abstract

Metabolic syndrome, insulin resistance, prediabetes, and overt type 2 diabetes mellitus are associated with an accelerated atherosclerosis (atheroscleropathy). This quartet is also associated with multiple metabolic toxicities resulting in the production of reactive oxygen species. The redox stress associated with these reactive oxygen species contribute to the development, progression, and the final fate of the arterial vessel wall in prediabetic and diabetic atheroscleropathy. The prevention of morbidity and mortality of these intersecting metabolic diseases can be approached through comprehensive global risk reduction.

Introduction

Metabolic syndrome (MS), insulin resistance (IR), prediabetes (PD), and overt type 2 diabetes mellitus (T2DM) amplifies and accelerates the risk of atherosclerosis with its associated effect on morbidity and mortality.

The multiple toxicities of this quartet: MS, IR, PD which includes impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), and overt T2DM result in accelerated atherosclerosis (macrovascular disease) or atheroscleropathy in addition to microvascular disease. It is appropriate to set forth definitions for this discussion.

Definition

Working definition of atherosclerosis:

Atherosclerosis is a systemic dysfunctional endothelial, focal occurring, chronic inflammatory, fibroproliferative, angiogenic, prothrombotic, multifactorial disease of the arterial intima caused by the retention of modified low density lipoproteins, hemodynamic, and redox stress [1–4] (figure 1) (figure 2).

Working definition of atheroscleropathy:

Atherosclerosis: The term used to describe the unique accelerated atherosclerosis observed in and associated with MS, IR, PD, and overt T2DM.

Henceforth, in this review the term atheroscleropathy shall be used to describe accelerated atherosclerosis associated with MS, IR, PD, and overt T2DM.

Three quarters of a century ago, a quote from Elliott P Joslin’s presentation to the American College of Physicians in 1927 seems appropriate.
Figure 1
An anatomical pull out image of each layer of the arterial vessel wall. The intima colored light blue is the location of the primary remodeling including the positive outward and later in time the negative inward remodeling with encroachment of the lumen. The inward negative remodeling is associated more with stable angina and stable plaques. The positive outward remodeling is associated more with unstable coronary syndromes and unstable VPs and has a much higher level of MMP-3 (stromelysin-ase) activity which may be increased as is MMP-9 in the diabetic patients. It is the MMPs which allow the tearing down (remodeling) in order for the outward remodeling to occur. See section on Redox Stress and MMP activity.
Figure 2
This image portrays the anatomical relationships of the endothelium, intima, media, and adventitia. Each of these layers play an important role in the development of atheroscleropathy. Since the discovery of the essential role of an intact endothelium for the vasomotor control of musculo-elastic arteries by Furchgott and Zawadski in 1980, and the discovery of the NOSs in 1989, the endothelium has been found to play a central role in the maintenance of healthy arteries and found to be placed in a central role for the development and progression of atherogenesis and subsequent atheroscleropathy. The endothelium is five times the weight of the heart and equal to the weight of the liver. This organ is placed at a critical location as an interface with nutrients and toxic products not only at its luminal side of musculo-elastic arteries but also at the endothelial extracellular matrix interface at the site of capillaries. This exciting monolayer of unique cells is responsible for the production of a gas NO that acts to modulate blood flow and is a naturally occurring interfacing antioxidant capable of scavenging ROS. The intima, sandwiched between the internal elastic lamina of the medial smooth muscle cell layer and the endothelium is the site of atherosclerosis, intimopathy, and the atheroscleropathy associated with MS, IR, PD, and T2DM. The injurious stimuli depicted on the luminal side of the endothelial cell (including redox and oxidative stress with ROS) result in the adaptive changes in which we are familiar: Remodeling of the arterial vessel wall: From Atheroma to Atherosclerosis, to Atheroscleropathy: A MALIGNANT TRANSFORMATION.
"I believe the chief cause of premature development of arteriosclerosis in diabetes, save for advancing age, is due to an excess of fat, an excess of fat in the body, obesity, an excess of fat in the diet, and an excess of fat in the blood. With an excess of fat diabetes begins and from an excess of fat diabetics die, formerly of coma, recently of arteriosclerosis."[5] Refer to A-FLIGHT toxicities sections (F), (L), (T).

This master clinician of diabetes was one of the first physicians to make the association regarding the double jeopardy of type 2 diabetes mellitus and atherosclerosis with its associated morbidity and mortality in cardiovascular disease.

Recognition of the prophetic view of Joslin has now been fulfilled in the 2001 National Cholesterol Education Program Adult Treatment Panel III guidelines. Diabetes (both T1DM and T2DM) is now considered a coronary risk equivalent and the metabolic syndrome is included in the multiple risks factors for the development of atherosclerosis. [6]

For today's atherosclerologists the history of atherosclerosis is rich and the theories are legion. Even today, knowledge in this field of study is expanding exponentially. In this review, we will try to remain focused on intimal redox stress and how this interacts with the manifold toxicities of IR, MS, PD, and T2DM to result in a unique accelerated atherosclerosis which shall be called atheroscleropathy.

Redox homeostasis, redox stress, and oxidative stress

Cellular respiration (the transference of electrons between oxygen species) allows each of us to survive on this planet not only at the cellular level but also as an organism. Homeostasis is a key element to all healthy physiologic functions throughout the body and when there is loss of homeostasis, there is usually disease.

Redox homeostasis describes the normal physiologic process of reduction and oxidation in order to re-pair unstable, damaging, reduced, reactive oxygen species (ROS) which will include the following oxygen free radicals (O2’ – superoxide, H2O2 – hydrogen peroxide, -OH’ hydroxyl radical, and singlet oxygen) and organic analogues which include reactive nitrogen species (RNS) primarily peroxynitrite ONOO’. This homeostatic balance between ROS and antioxidant capacity is in contrast to redox stress (redox imbalance) which implies a loss of this unique homeostasis resulting in an excess production of ROS (redox imbalance) either through the process of reduction or oxidation.

Oxidative Stress implies a loss of redox homeostasis (imbalance) with an excess of ROS by the singular process of oxidation. Both redox and oxidative stress may be associated with an impairment of antioxidant defensive capacity as well as an overproduction of ROS.

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 sulfhydryl rich proteins (leading to stiff aged proteins specifically collagen of the extracellular matrix). [7]

The evolutionary process of redox homeostasis allows humans to survive in an atmosphere of high oxygen content. In addition our bodies have become "hard wired" to uti
Table 2: Courtesy [9] Origins of reactive oxygen species (ROS) and cellular location

<table>
<thead>
<tr>
<th>Nicotinamide adenine dinucleotide reduced (NADH)</th>
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<tbody>
<tr>
<td>NADH Oxidase NADH / NAD⁺ (mitochondrion, cytosol)</td>
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<tr>
<td>NADH + 2O₂ → NAD⁺ + H⁺ + 2O₂⁻ (Super Oxide)</td>
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<tr>
<th>Nicotinamide adenine dinucleotide phosphate reduced (NAD(P)H)</th>
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<tr>
<td>NAD(P)H Oxidase NAD(P)H / NAD(P)⁺ (membrane)</td>
</tr>
<tr>
<td>NAD(P)H + 2O₂ → NAD(P)⁺ + H⁺ + 2O₂⁻ (Super Oxide)</td>
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**Super oxide dismutase (SOD):**
- MnSOD = Mitochondrial SOD
- CuZnSOD = Intracellular (cytosolic) SOD
- EcSOD = Extracellular SOD

<table>
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<th>O₂⁻ + SOD → H₂O₂ (hydrogen peroxide)</th>
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<tr>
<td>Fenton Reaction: H₂O₂ + Fe³⁺ → •OH (the hydroxyl radical) + Fe²⁺ + OH⁻</td>
</tr>
<tr>
<td>Haber-Weiss Reaction: H₂O₂ + O₂⁻ → •OH⁻ (the hydroxyl radical) + O₂⁻+OH⁻</td>
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**Peroxynitrite: origins of reactive nitrogen species (RNS):**
O₂⁻ is consumed. Nitric oxide (NO) is also consumed in this process with the creation of reactive nitrogen species (RNS).

| O₂⁻ + NO → ONOO⁻ (peroxynitrite) + tyrosine → nitrotyrosine |
| O₂⁻ + NO → ONOO⁻ (peroxynitrite) + arginine → nitroarginine |

Nitroarginine competes for arginine in the formation of eNO.

Nitrotyrosine reflects redox stress and leaves an indelible measurable footprint.

NO: the good; O₂⁻: the bad; ONOO⁻: the ugly [122,9]

Table 3: Courtesy [8,9] The manifold toxicities of insulin resistance, metabolic syndrome and T2DM

<table>
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<th>A-FLIGHT toxicities</th>
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<tr>
<td>A</td>
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<tr>
<td>Ang II (also induces PKC)</td>
</tr>
<tr>
<td>AGEs/AFEs (advanced glycosylation/fructosylation endproducts)</td>
</tr>
<tr>
<td>Antioxidant reserve compromised</td>
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<tr>
<td>Absence of antioxidant network</td>
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<tr>
<td>Ageing</td>
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<tr>
<td>Angiogenesis (induced redox stress) Arteriogenesis (impaired PAI-1)</td>
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<tr>
<td>Atherosclerosis – Atheroscleropathy. [ROS beget ROS]</td>
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<td>I</td>
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<tr>
<td>Inflammation toxicity</td>
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<td>G</td>
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<tr>
<td>Pseudohypoxia (NADH/NAD increased)</td>
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<td>t homocysteine toxicity</td>
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See reference [8,9]
lize the mechanism of redox stress injury to fend off invading infectious organisms and survive our environment.

Paradoxically, (when there is loss of homeostasis resulting in redox or oxidative stress) this protective mechanism turns on our own cells; tissues and causes damage, especially the intima in the atheroscleropathy associated with MS, IR, PD, and overt T2DM. This constellation of MS, IR, PD, and T2DM is associated with an elevated tension of redox stress within the intima (also the islet in MS, IR, PD, and T2DM) due to multiple toxicities. (table 3) Each of these A-FLIGHT toxicities result in the formation of damaging ROS. [8,9]

Not only are ROS involved in the development of type 1 diabetes mellitus (T1DM) and T2DM but also play an important role in the long-term development of the associated complications: The multiple diabetic-opathies (A – DINNER: atheroscleropathy, angiogenesis (accelerated) and arteriogenesis (impaired), diabetic cardiomyopathy and dermopathy, intimopathy, nephropathy, neuropathy, enteropathy, retinopathy (table 4). This review will focus primarily on the association of redox stress in the intima and how it interacts with MS, IR, PD, and T2DM.

**Metabolic syndrome and insulin resistance**

IR describes the condition whereby there is a resistance to insulin mediated glucose uptake by cells and is central to the clustering of multiple metabolic abnormalities and clinical syndromes (figure 3). The clustering phenomenon was first described by Kylin in 1923 when he described the clustering of three clinical syndromes: hypertension, hyperglycemia, and hyperuricemia. [10]

In 1936 Himsworth [11] noted that a large number of diabetic patients were insulin insensitive and suggested that diabetics be divided into groups that were insulin sensitive and insulin insensitive.

Yalow and colleagues in 1965 [12] were first to discover an insulin assay and reported that IR was a condition in which insulin does not produce the same glucose lowering effects seen in insulin-sensitive individuals.

These concepts were rejuvenated and immortalized by Reaven in 1988 given as the Banting lecture. [13] The clustering phenomenon has gone by many names since Dr. Reaven first described the metabolic and clinical associations of the many names of Syndrome X. (table 5)

By 1999, the World Health Organization had chosen a unifying definition for this syndrome of many names and elected to use the term metabolic syndrome rather than the insulin resistance syndrome because they felt it was not well established that insulin resistance was the cause of all components of the syndrome. [14]

Additionally, there are at least a dozen factors that link clinical suspicions to the metabolic syndrome. (table 6) Factors and findings in this syndrome occur together all to frequently to be considered a coincidence and there are common underlying factors that may explain this coexistence. Namely, the well documented hyperinsulinemia story and the more recent hyperamylinemia and amylin derived islet amyloid story. [8,9]

MS affects approximately 47 million or greater Americans. [15] Of these 47 million, only 20% will develop T2DM and the remaining 80% will be able to compensate (at least for a period of time) through the process of beta cell

### Table 4: Multiple diabetic-OPATHIES.

| A | Atheroscleropathy. Angiogenesis (induced). Arteriogenesis (impaired). |
| D | Dermopathy: Diabetic Scleroderma – Stiff skin, vulcanization of collagen (cross-linking). Necrobiosis Lipoidica Diabeticorum. Brown sunken atrophic scars anterior shin of legs (impaired scar formation associated with increased levels of PAI-1 and impaired injury remodeling). |
| N | Nephropathy. Microvascular. |
| N | Neuropathy. Microvascular. Overriding sympathetic stimulation pro vasoconstrictive. |
| E | Enteropathy. Delayed gastric emptying. |
| R | Retinopathy. Microvascular. |

Observations from Table 4: Intimopathy and atheroscleropathy are very similar. Similar shared glucotoxicity in T1DM and T2DM. Dissimilar toxicities in that the toxicities of MS, IR, PD and the role of the aging process predate the development of overt T2DM. See reference [8,9]
expansion, hypertrophy, and hyperplasia (utilizing the replicative pool of periductal cells). [16,17]

The resulting hyperinsulinemia, hyperamylinemia (37.6 million = 80% of 47 million) does not come without a price to pay as this compensatory mechanism places these patients at risk for hypertension, atheroscleropathy, and subsequent coronary artery disease. [18,19] (figure 3) See section (A). Ang II, (A). Amylin toxicity, and (I). Insulin toxicity.

The manifold – A-FLIGHT toxicities
(A). Angiotensin II toxicity
Angiotensin II (Ang II) is associated with hypertension, MS, IR, PD, and T2DM both systemically and at the local tissue level. Currently, there is evidence that a local tissue renin angiotensin aldosterone system (RAAS) is operative within the intima and islet as angiotensin type one (AT-1) receptors have been identified as being present on smooth muscle cells, endothelial cells, and the beta cells within the intima and islet [20,21] Insulin is known to upregulate the AT-1 receptor [22] and there exists cross talk between the insulin and the Ang II signaling systems [23] In 1995, Copper et al. were able to demonstrate that amylin activates the RAAS with elevations in renin and aldosterone in humans [24] and, in 2001, Ikeda et al. were able to demonstrate that insulin, proinsulin and amylin infusions resulted in significant increases in renin release and that proinsulin and amylin enhanced this insulin-stimulated renin release in the perfused rat kidney [25].

Taken together, these data support the strong influence of a local RAAS mechanism operating within the intima and islet for the local production of excess Ang II. The islet is

Figure 3
The cluster of multiple abnormalities are associated with the metabolic syndrome and insulin resistance is central to the development of ATHEROSCLEROPATHY, cardiovascular disease, and events in the patient with MS, IR, PD, and overt T2DM.
The vascular NAD(P)H oxidase enzyme to come into play. Ang II is one of the most potent endogenous stimuli for the generation of superoxide O$_2^-$ via the activation of vascular NAD(P)H oxidase. [26,27]

The interruption of this mechanism by the angiotensin converting enzyme inhibitor (ACEi) ramipril in the Heart Outcomes Prevention Evaluation (HOPE) study may help to explain the 32% risk reduction for developing T2DM as well as the dramatic reduction in cardiovascular events. [28]

A special reference to Griendling and Harrison seems appropriate: "Out, damned DOT! Out I say" (where the damned DOT represents the unpaired dots on Lewis diagrams). [29] One of the best ways to prevent these dots from forming is to prevent excess substrates (table 3) which cause the multiple toxicities and the multiplicative effect of the A-FLIGHT toxicities associated with MS, IR, PD, and T2DM.

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In MS, IR, PD, and T2DM the intima and islet milieu will be laden with the necessary substrates (hyperinsulinemia, hyperproinsulinemia and hyperamylinemia) to activate the damaging cascading mechanism of Ang II, NAD(P)H oxidase, superoxide (O$_2^-$) and peroxynitrite (ONOO$^-$) production while consuming the natural endogenously produced antioxidant nitric oxide (NO) within the vulnerable intima and islet.

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(A). Advanced glycosylation endproducts: AGE

Advanced glycosylation endproducts (AGEs) are formed as a result of the non-enzymatic damaging protein glycation due to an excess of glucose (hyperglycemia) present in both T1DM, PD, and T2DM. AGEs are initially formed through the process of a glucose nucleophilic addition reaction with proteins forming a Schiff base followed by the formation of an Amadori compound which undergoes further reactions, rearrangements, dehydrations and cleavage resulting in brown insoluble, cross linked complexes called AGEs. This process is thought to liberate H$_2$O$_2$ through two pathways: the first is the 1,2-enolization pathway which leads to 3-deoxyglucosone forming H$_2$O$_2$ and glucosone; the second pathway is the 2,3-enolization pathway leading to 1-deoxyglucosone and putative 1,4-deoxyglucosone. Under oxidative conditions, the 2,3-enediol is thought to generate H$_2$O$_2$ and carbamoylmethyllysine. 3-deoxyglucosones are known to be both highly reactive intermediates in non-enzymatic glycosylation and also potent cross-linkers which are responsible for the polymerization of proteins to AGEs. These highly cross-linked proteins, especially collagen, cause a stiffening within the vessel which results in decreased compliance of the arterial vessel wall and may well play an important role in the development of diabetic diastolic dysfunction, diabetic cardiomyopathy, and the diastolic dysfunction of the arterial vessel wall. Furthermore, there are advanced fructosylation endproducts (AFEs), which actually have a greater affinity binding to proteins than glucose and follow a similar pattern in the production of the ROS. [30–33] An excellent in depth review of AGE can be found in an article by Aronson and Rayfield where they discuss how hyperglycemia promotes atherosclerosis [34].

The multiligand immunoglobulin superfamly cell surface receptor: the receptor for advanced glycation endproducts (RAGE) is up-regulated by the presence of AGE and results in the signal transduction of nuclear factor kappa B (NFkappa B) which then results in a chronically active inflammatory state and links this section to section (I). Inflammation Toxicity and atherosclerosis. [35,36]

(A). Antioxidant enzymes

Antioxidant reserve compromised

In addition to the excess generation of the ROS seen in diabetes, there exists an impaired generation of endogenous antioxidants. Superoxide dismutase (SOD), [37] glutathione reduced (GSH), [38] and ascorbic acid (Vitamin C) [39] are all decreased and associated with atherosclerosis in diabetes. Moreover, there is evidence of the diminished capacity of other antioxidants such as uric acid and vitamin E with a reduced activity of catalase and glutathione peroxidase (GPx). (Table 6) [40] The exact mechanisms of impairment are still not completely understood but two explanations exist. Protein glycation may be a
mechanism that damages the protein within the primary antioxidant enzymes, and the antioxidant enzymes which are co-dependent on one another, may be dysfunctional if one or the other is being consumed by an overactive demand such as compromised GSH function due to the depletion of NADH in the polyol pathway.

It seems quite logical that both mechanisms may be in play at one time or another in the diminished antioxidant defense mechanisms. Another example is glutathione disulfide (GSSG) which is reduced to GSH at the expense of NAD(P)H. [41]

Absence of network antioxidant enzymes
eNOS
The absence of network antioxidant enzymes could play an additional role. A good example of this condition would be the endothelial nitric oxide synthase (eNOS) -/- knockout mouse model by Duplain and Scherrer.

They were able to demonstrate that insulin resistance, hyperlipidemia, and hypertension were present in mice lacking the specific isoform eNOS. This implicates eNOS not only in the endothelial cell (important in the regulation of arterial pressure) but also in the loss of its expression in skeletal muscle which impairs insulin stimulated glucose uptake, and that its loss (both at the endothelial and skeletal muscle sites) impairs lipid homeostasis and creates insulin resistance. [42] This represents the loss of the naturally occurring free oxygen radical scavenging antioxidant effect of endothelial nitric oxide (eNO) (Table 7). Does this apply to humans?

There is evidence of a gene polymorphism in humans and recently Miyamoto et al.[43] were able to demonstrate that a gene polymorphism, Glu298Asp in exon 7 of the eNOS gene, was associated with coronary spastic angina and myocardial infarction and found further evidence for this gene polymorphism in the statically significant association with the development of essential hypertension in two separate Japanese populations. There could be other gene polymorphisms in other populations as well as in other antioxidant genes that relate to insulin resistance, metabolic syndrome, and hypertension. As the human genome evolves, we are certain to find other alterations in various populations throughout the world.

Asymmetrical dimethylarginine (ADMA) has recently been shown to be associated with endothelial dysfunction and increased risk of cardiovascular disease. Stuhlinger, Reaven, Tsao, and colleagues were able to demonstrate a positive correlation with impaired insulin-mediated glucose disposal and elevated levels of ADMA. Plasma ADMA concentrations increased in insulin-resistant subjects independent of hypertension. Increases in plasma ADMA concentrations may contribute to the endothelial dysfunction observed in insulin-resistant patients.

Elevated levels of ADMA have been observed in IR, hypertension, hyperlipidemia, hyperglycemia, hyperhomocysteinemia, and renal failure. ADMA is formed by protein arginine N-methyltransferases and LDL-C both native and oxidized up regulates PRMT’s increasing ADMA. [44,45]

Under physiologic homeostatic conditions, eNOS is the endothelial constitutive (rate limiting) enzyme responsible for the conversion of L-arginine to NO and L-citrulline. It requires a cofactor tetrahydrobiopterin (BH4). There is a paradoxical uncoupling of the eNOS enzyme that allows this above reaction to be capable of producing superoxide (O2') if there is insufficient BH4, L-arginine, or if there is direct interference with and/or defect in the eNOS enzyme. Uncoupling of eNOS enzyme results in the production of damaging O2' adding to the oxidative stress within the arterial vessel wall.
Causative factors for eNOS uncoupling are as follows: Increased O2' and ONOO', elevations in glucose and both native LDL-C and oxidatively modified, mmLDL-C, hyperhomocysteinemia see section (H), decreased or impaired Cofactor BH4, decreased L-arginine, increased ADMA, Hcy, and CRP. Each of these factors can contribute to making the endothelium of the patient with MS, IR, PD and overt T2DM a net producer of [O2']. The above dysfunctional endothelium with a decreased ratio of NO / ROS. RNS can further contribute to the overall increase in intimal redox stress and atherosclerosis.

The ROS stemming from the A-FLIGHT toxicities additionally play a role in the competitive inhibition of eNOS. Redox stress results in the production of nitroarginine as well as nitrotyrosine. Nitroarginine then competes with L-arginine as a substrate for eNOS to generate NO. Stepp and colleagues were able to demonstrate a 4 fold increase in O2' and an 8 fold increase in O2' when endothelial
cells were exposed to native LDL-C and mmLDL-C respectively. This uncoupling of eNOS plays an important role in endothelial cell dysfunction and increased oxidative stress. [47] Hyperglycemia and peroxynitrite (ONOO') also induce eNOS uncoupling with increases in O₂' production. [48] Just published, Verma S and colleagues reported that CRP caused a marked down regulation of eNOS mRNA and protein expression with subsequent lower eN0 production. The authors point out that CRP may not just be a marker of atherosclerosis and increased risk of acute coronary events, but may also be a mediator of this disease process. Strategies designed to lower CRP may reduce cardiovascular risk by directly improving bioavailability of NO and endothelial function. [49] See section (I). Inflammation.

There are undoubtedly many more scenarios in which eNOS can be impaired with resulting decreased NO but at this point in time it is certainly interesting to see a tight connection of impaired eNOS and the MS, IR, PD, and T2DM. Additionally, the synergistic importance regarding elevations of both glucose and native LDL-C or mmLDL which result in elevations of the detrimental superoxide (O₂') can uncouple the eNOS enzyme leading to even further increases in O₂'. The importance of treating LDL-C, HbA1c, and hypertension to goal are therefore paramount in reducing the oxidative damage and endothelial cell dysfunction. (table 9) (figure 6) These examples only strengthen the statement that ROS beget ROS.

The synergism and the vicious cycle of redox and oxidative stress to the arterial vessel wall from ROS produced by vascular cells, especially the endothelium, as a result of the A-FLIGHT toxicities necessitates an aggressive global approach. Wong T.Y. and colleagues for the Atherosclerosis Risk In Communities (ARIC) Investigators were able to show that retinal arteriolar narrowing was independently associated with the risk of developing future diabetes. This supports a microvascular role in the development of clinical diabetes and provides clinical evidence to support a hypothesis that eNOS and endothelial dysfunction may be implicated in the pathogenesis of diabetes. This new clinical information, of arteriolar narrowing preceding the clinical onset of diabetes and implicating endothelial cell dysfunction (including an eNOS defect) could play a major important role in the development of this polygenic – multifactorial disease of MS, IR, PD, overt T2DM and atheroscleropathy. [32]

This leads to an interesting Hypothesis:

Could it be that T2DM is really a cardiovascular disease (evolving around a primary eNOS enzyme dysfunction or defect with an effect on MS and IR) with a late manifestation of glucose elevation i.e. PD and overt T2DM? This would certainly tie the natural history of T2DM and atheroscleropathy together [53].

Other antioxidant enzymes
If any one of the antioxidant enzymes (table 7) is missing or impaired or any combination of them are impaired, then we would expect to see a similar event as in the knockout mouse model. It would not have to be a complete knockout of the enzyme, as discussed above, as various gene polymorphisms could exist which could result in a decreased antioxidant reserve.

(A). Ageing
Ageing has been shown to be associated with an increased risk of developing T2DM and atherosclerosis. Ageing allows the multiplicative effects of the A-FLIGHT toxicities to become manifest. Advanced ageing leads to impaired endothelial nitric oxide synthesis and also enhanced endothelial apoptosis.

In addition, aged cells have a significantly enhanced concentration (more than 3 fold) of oxidized low density lipoprotein, TNFalpha and caspase-3 activity as compared to young cells. The decrease in eN0 associated with aged cells creates a deficiency of the naturally occurring antioxidant eNO. [54]

Similarly, excess redox stress is felt to contribute to ageing. Information on the relationship of redox stress and ageing and inflammation (see section "(I). Inflammation Toxicity") is rapidly increasing and gaining wider recognition. [55,56]

(A). Amylin toxicity
Amylin, also termed islet amyloid polypeptide (IAPP) is a 37 amino acid polypeptide co-synthesized, co-packaged, and co-secreted by the islet beta cell with insulin. It may be considered insulin’s a fraternal twin.

Amylin parallels insulin synthesis, secretion, and excretion so that whenever you have hyperinsulinemia you have hyperamylinemia and, in the same way, when insulin levels decline amylin levels decline.

Amylin stimulates lipolysis in vivo and may be a possible mediator of induced insulin resistance. Ye et al., were able to demonstrate that amylin infusion (5 nmol/h for 4 h) conscious rats that fasted for 5-7 hours resulted in an elevation of insulin, lactate and glucose (P < 0.05 vs. control).

Despite the rise in insulin, plasma non-esterified fatty acid and glycerol were also elevated (P < 0.001). Although the plasma triglyceride content was unaltered, the triglyceride content in the liver was increased by 28% (P < 0.001) with
a similar tendency in muscle (18%, \( P = 0.1 \)). These effects were blocked by the rat amylin antagonist amylin-(8–37) and also by the anti-lipolytic agent acipimox. The authors concluded that amylin could exert a lipolytic-like action in vivo. [57]

This elevation in amylin would correspond to the insulin resistant state with associated elevation in amylin in humans. These data indicate that amylin may play a role by elevating free fatty acids which would aggravate or induce the underlying insulin resistance and provide a mechanism for increasing the free fatty acid substrate for increased redox stress, cytotoxicity and intimal remodeling associated with atheroscleropathy.

There are amylin binding sites within the renal cortex and amylin activates the RAAS with elevations in renin and aldosterone [24]

**Figure 5**
Vulnerable plaques are proinflammatory, profibrotic, prothrombotic, proangiogenic, lipid laden and recently found to be acidic. The activated endothelium is associated with endothelial dysfunction due to eNOS dysfunction and eNOS uncoupling with resultant overproduction of superoxide \([O_2^*]\). Diabetes is associated with a dysfunctional endothelium not only at sites of vulnerable plaque but have been shown to be a systemic net producer of \([O_2^*]\). The inflammatory cells and intimal remodeling are depicted in this image as well as an intraplaque hemorrhage (IPH) from the angiogenic vasavasorum vessels originating from the adventitia of this lipid laden vulnerable plaque. Redox stress and ROS play a prominent role within the intima. The profibrotic arm of the unstable VP is responsible for the positive outward and negative inward remodeling.
These findings suggest that glucotoxicity resulting in AGE formation both promotes and accelerates redox stress. [58]

Janson et al. have found that intermediate sized toxic amyloid particles (ISTAPs) have been found to be cytotoxic to beta cells inducing apoptosis by membrane disruption. [59]

(A). Angiogenesis (accelerated); Arteriogenesis (impaired); Vascularization Paradox In T2DM

The process of Angiogenesis starts with capillaries and ends with more capillaries

As the atheroma matures there is an associated intense plaque angiogenesis arising primarily from the adventitial vasa vasaorum (atherosclerotic intimopathy). These vessels invade the arterial vessel wall in a malignant like fashion.

This plaque vascularization (angiogenesis) corresponds to the presence of the inflammatory infiltrate at the shoulder of these lesions, the development of the large lipid core, the thin fibrous cap, and the decrease in SMCs within the fibrous cap, to form, what we now term the vulnerable plaque. [1–4]

Extensions of the vasa vasaorum (the vessels within a vessel) act as a custom delivery system within the vessel walls' vulnerable shoulder region supplying: 1. substrates of the RAAS; 2. substrates of native LDL-C; 3. the second wave of inflammatory cells; 4. inflammatory mediators (various cytokines and growth factors); and 5. provide an addition-
al conduit and source of redox stress at the endothelial cell – extracellular matrix interface within these vulnerable plaques.

This process is accelerated in the MS, IR, PD, and T2DM as well as the T1DM patient. Vasa vasorum derived fragile capillary-like vessels are prone to rupture and create intra-plaque hemorrhages (IPH) which destabilize the plaque and promote the possibility of being prone to rupture with ensuing cardiovascular events. The pseudohypoxia (increased ratio of NADH/NAD+ discussed by Williams-son and Kilo see section (G) glucotoxicity.) in the polyol-sorbitol pathway as well as the true hypoxia induced by the increasing intima media thickness may act to induce the nuclear hypoxia inducible factor (hif-1) within the smooth muscle and endothelial cells which result in the increased expression of vascular endothelial growth factor (VEGF) which is so central and vital to the angiogenic process. This diabetic atherosclerotic intimopathy (plaque angiogenesis) would be akin to the diabetic retinopathy. [3,60–62]

<table>
<thead>
<tr>
<th>Enzymic antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPER OXIDE DISMUTASE (SOD) – Location: mitochondrion</td>
</tr>
<tr>
<td>MnSOD (mitochondrial)</td>
</tr>
<tr>
<td>CuZnSOD (intracellular)</td>
</tr>
<tr>
<td>CATALASE – Location: peroxisome</td>
</tr>
<tr>
<td>(Glutathione peroxidase) [GSH + H2O2 – OH + GSSG]</td>
</tr>
</tbody>
</table>
| (Glutathione reductase) [GSSG – GSH] at the expense of [NADH – NAD+] |}

*eNOS (nitric oxide synthase), – Location: membrane

Isoforms:
(e) NOS (endothelial): good (importance of eNOS uncoupling) LDL native and oxidized. (n)NOS (neuronal): good (i)NOS (inducible-inflammatory): good in host defense. BAD in chronic inflammation. O2− and nitric oxide (NO) are consumed in this process with the creation of reactive nitrogen species (RNS). O2− + NO – ONOO− (peroxynitrite) + tyrosine → nitrotyrosine. (also causes eNOS uncoupling)

Nitrotyrosine reflects redox stress and leaves a measurable footprint. NO: the good; O2−: the bad; ONOO−: the ugly [122]

*eNOS uncoupling causes the generation of O2' instead of NO induced by LDL-C, Glucose, O2', and ONOO'.

Nonenzymic antioxidants

| URIC ACID |
| VITAMIN A |
| VITAMIN C |
| VITAMIN E |
| THIOLS |
| APOPROTEINS: Ceruloplasmin and transferrin. Bind copper and iron in forms which cannot participate in the Fenton reaction. [9] |

The process of arteriogenesis starts with small arterioles and ends with larger arterioles

In contrast the vascularization process of arteriogenesis is impaired. Even though patients with diabetes (both T1DM and T2DM) have a much higher number of atherosclerotic diseased arteries, mean coronary collateral vessels (CCV) are significantly decreased. [63]

Elevations in plasminogen activator inhibitor-1 (PAI-1) are present in the MS, IR, PD, and T2DM patient. Remodeling collateralization (arteriogenesis) is stimulated by the tissue and urokinase plasminogen activators (tPA and uPA). PAI-1 elevations decrease the conversion of plasminogen to plasmin because it inhibits tPA and uPA. As plasmin is impaired there is a reduction of the conversion of inactive or pro-MMP-1 to active MMP-1 with inhibition of ECM turnover with resultant impaired CCV formation. This paradox in diabetes vascularization contributes greatly to the known poor outcomes associated with cardiovascular events in the diabetic. [3]

(A). Atherosclerosis and atheroscleropathy

Once atherosclerosis and atheroscleropathy have been initiated and sustained, this process is self – perpetuating
(self sustaining) and the vicious cycle of ROS begetting ROS comes into play with all of its inherent complications. It’s as if the multiple A-FLIGHT toxicities polymerize with redox stress and its associated ROS acting as an accelerant. The associated ischemic cardiomyopathy and the distinct diabetic cardiomyopathy are not within the scope of this review.

**F. Free fatty acids**

Free fatty acid (FFA) elevation is known to be associated with IR, MS, PD, and T2DM. The metabolically active form of FFAs are cytosolic long-chain acyl-CoA esters (LCACoA) and are responsible for cytosolic neutral triglyceride deposition in adipose and non-adipose tissues.

In 2001, McGarry gave an excellent presentation at the American Diabetes Association meeting (ADA 2001 Banting Lecture), discussing in detail how toxic FFA and LCACoA may be important in the development of insulin resistance, progressive beta cell dysfunction and death associated with T2DM. [64]

Central obesity is associated with increased cytosolic neutral fat triglyceride stores in adipose and non-adipose tissues such as muscle (skeletal and cardiac), the liver, pancreatic beta cells and, possibly, endothelial cells. [64,65]

Intra-myocellular lipid was found to be more highly correlated with insulin resistance than any other commonly measured indices such as body mass index, waist-to-hip ratio or total body fat. Low insulin sensitivity was accompanied by a marked increase in intra-myocellular lipid. Bakker et al.[65] proposed that the chronic low-grade production of ROS produced by respiring mitochondria is enhanced by excessive cytosolic triglyceride stores and LCACoA esters in non-adipose tissue.

They proposed that LCACoA esters exert an inhibitory effect on the adenosine nucleotide translocator with a resultant decrease in the ADP available. This decrease in ADP slows the flow of electrons along the electron transfer chain and increases the possibility of having single unpaired electrons to create the superoxide anion (O$_2^-$) increasing oxidative mitochondrial stress, thus resulting in a dysfunctional cell. Moreover, they suggest that these phenomena not only accelerate the atherosclerotic process but also induce endothelial dysfunction and microalbuminuria prior to the development of T2DM and possibly beta cell dysfunction and failure. [65]

It is difficult to completely separate FFA toxicity from the sections which follow on lipoprotein toxicity and triglyceride toxicity as there is a dynamic relationship between these three in the A-FLIGHT toxicities. In fact, FFAs are transported by the protein fraction, albumin, and lipases are constantly removing the long chain fatty acids from the glycerol backbone of triglycerides at the interface of the capillary endothelial cells creating free fatty acids which can freely move into cells throughout the body. Intracellularly, the FFAs are then added to the glycerol backbone in order to form cytosolic triglycerides stored as neutral fat, or are oxidized for fuel and energy generating ATP. If mitochondrial beta oxidation is over utilized or dysfunctional, the excess may then undergo the toxic non-beta non-mitochondrial pathway generating toxic FFAs or ceramide (see section "L. Lipotoxicity – Specific").

**L. Lipotoxicity – generalized**

Lipotoxicity promotes oxidative stress and is associated with MS, IR, PD, and T2DM. There is an associated defect of lipoprotein metabolism frequently referred to as the "lipid triad". Elevated VLDL or triglycerides, atherogenic small dense LDL, and decreased HDL comprise this triad which is associated with atherosclerosis and coronary heart disease as well as increased redox stress. [66–68]

The increased VLDL, triglycerides, atherogenic small dense LDL cholesterol and the diminished amount of the anti-atherogenic, antioxidant anti-inflammatory high density lipoprotein cholesterol would reduce the natural antioxidant reserve. This combination supports an increase in redox stress in addition to the previously discussed FFA toxicity. This also tends to support the oxidation, glycation and glycoxidation of existing lipoproteins (modification) which results in increased ROS and redox stress.

Lipoproteins have the function of transporting lipids throughout the body. Low density lipoproteins are responsible primarily for the transport of cholesterol with the protein moiety involved: apolipoprotein (Apo) B 100. Very low density lipoproteins are responsible for the transport of triglycerides with the protein moiety involved: Apo E. High density lipoproteins are responsible for reverse cholesterol transport and play an important role in being a naturally occurring potent anti-inflammatory and antioxidant agent with the protein moiety involved: Apo A. It is the protein moiety of the lipoproteins that is modified by the processes of oxidation, glycation, and glycoxidation with a resultant increase in redox stress and the production of ROS. Furthermore, the modification of the protein moiety is responsible for their retention within the intima, inducing atherogenesis and thus atherosclerosis. [69,70]

**L. Lipotoxicity – specific**

Lipotoxicity is also associated with MS, IR, PD, and T2DM. Unger et al.[71–76] feel this specific lipotoxicity is attributed to products of the excessive non-beta- (non-mi-
tochondrial) oxidative metabolism of FFA in the skeletal and the myocardial muscle, the liver and the pancreatic islets.

In addition, these toxic metabolic products are thought to cause the complications of MS, IR, PD, and T2DM by creating cellular dysfunction and, in time, promoting programmed cellular death (lipoapoptosis). [74,75] In the normal state, FFA delivery to non-adipose tissue is closely regulated to its need for fuel. FFAs normally rise during exercise and fasting in order to meet metabolic requirements and thus, homeostasis is maintained. However, as a result of over-nutrition (western diet), the FFA influx may exceed FFA usage and FFA non-beta oxidation ensues.

These non-mitochondrial FFA metabolites, which are responsible for injuring cells, result in lipoapoptosis, include triglycerides, ceramide, and products of lipid peroxidation. Ceramide (an amino alcohol with a LCA-CoA attached to the amino group) has been implicated for some time in the apoptotic pathway of the T1DM autoimmune destruction of beta cells by sphingomyelin degradation. [77]

Ceramide can be formed in these cells by direct de novo synthesis from FFAs. [72] Ceramide is responsible for the induction and activation of NFkappa B. [78]

In the process of developing T2DM, only those beta cells with the highest fat content give way to the ceramide cascade thus leaving enough functioning beta cells to maintain insulin independence but not enough to compensate for the co-existing insulin resistance with the subsequent development of impaired glucose tolerance, impaired fasting glucose and the development of overt T2DM. This entire process is magnified and progresses due to an intense redox (oxidative stress within the islet and intima which incorporates and implicates the multiplicative manifold A-FLIGHT toxicities).

(I). Insulin toxicity
Insulin toxicity (hyperinsulinemia, hyperproinsulinemia, and hyperamylinemia) is associated with MS, IR, PD, and early T2DM. In late T2DM as beta cell failure develops there is no longer insulin toxicity. Insulin is known to up-regulate the number of AT-1 receptors, activate the RAAS, and be capable of cross talking with the AT-1 receptor. Recently, AT-1 receptors have been identified on the islet beta cell and the islet endothelial cell.

Thus, hyperinsulinemia can be linked back to the section “(A). Angiotensin II” with resultant increased redox stress systemically as well as within the intima and islet as insulin, proinsulin and amylin are all three elevated within the intima and islet milieu. [20–25]

Endogenous Hyperinsulinemia (eHI) is associated with MR, IR, PD, and early T2DM. Additionally, eHI is associated with hypertension and atheroscleropathy (coronary artery disease). eHI is also associated with elevated FFA, plasminogen activator inhibitor-1 (PAI-1), elevated sympathetic tone and activity, increased sodium and water re-absorption leading to volume expansion which leads to and supports hypertension in the clustering phenomenon of MS relating to section (I). Hypertension toxicity. Previously discussed, insulin, proinsulin, and amylin have been noted to contribute to the elevation of Ang II with increases in renin and aldosterone.

Amylin the fraternal twin of insulin has been shown to induce lipolysis which elevates FFA and links to the sections on (F), (L), and (T). The reader will note that the various sections within the A-FLIGHT toxicities interact and play off one another creating a vicious cycle of promoting redox stress within the intima and islet.

Additionally, it is important to note that increased proinsulin concentrations predict death and morbidity caused by coronary heart disease independent of other major cardiovascular risk factors. [79,80]

(I). Inflammation toxicity
A new insight into the study of atherosclerotic plaques has evolved over the past decade and now the accepted role of inflammation in vulnerable plaque pathology has been widely accepted in the field of atherosclerosis.

Currently, chronic inflammation is gaining momentum as a prelude to MS, IR, PD, and T2DM.

Increasingly, this quartet in the continuum of the natural history of diabetes is being accepted by diabetologists and researchers as a chronic inflammatory disease. [9,81–92]

The four "cardinal signs" of inflammation described by Aulus C. Celsus in De re medicina in 30 A.D. are: rubor, calor, dolor, and tumor and currently there is a large body of information that atherosclerotic vulnerable plaques (VP) fit the above criteria.

Rubor
VPs have a unique increase in angiogenesis of the vasa vasorum which act as a FedEx delivery system and thus increase flow for inflammatory cells and injurious substrates to promote vulnerability at the endothelial extracellular matrix interface.

Calor
Recently, these VPs have been shown to possess a higher core temperature.
Dolor
There is no direct pain associated with the VP, however, once it is ruptured the cardiovascular event is quite painful and the fixed stenotic lesions of atheroscleropathy create the painful syndrome of angina pectoris.

Tumor
There is no doubt that there is swelling of even the atheroma (outward positive remodeling) as well as encroachment upon the lumen with negative remodeling resulting in a stenotic lesion which entertains the fifth sign of inflammation, functio laesa, inhibited or lost function. Recently, Naghavi M et al. were able to show that these VPs were more acidic which may be an asset in detecting their presence in vivo. [93–96] (figure 5)

Inflammation toxicity (with increased redox stress and cytokines) is associated with MS, IR, PD, and early as well as late T2DM.

The innate inflammatory mediators, TNFalpha and interleukin 6 (IL-6), are tightly associated with central (visceral – omental) obesity, MS, IR, PD, and T2DM. [97][98,99] This innate immune system (IL-6 and TNF alpha which activates the acute phase response) is more ancient and does not require a previous antigenic stimulus as does the acquired antigen – antibody related immune system.

Downstream from IL-6 and TNFalpha, elevated white blood cell count, sialic acid, orosomucoids and the acute phase reactants: highly sensitive C reactive protein (hs-CRP), fibrinogen, and serum amyloid A are associated with the development of T2DM and atheroscleropathy. Factor VIII, von Willebrand factor (indicating endothelial cell activation) and activated partial thromboplastin time have also been implicated in the development of T2DM. [100]

NFkappa B is associated with redox stress and the isoform inducible iNOS in the apoptosis of the beta cell in both T1DM and T2DM. Both NFkappa B and TNFalpha are induced by ROS [60].

The adhesion of the leukocytes to the post-capillary venule is an important step in the inflammatory process and the adhesion of the leukocytes to the endothelial cells is induced by ROS. This effect is abolished by catalase but not SOD, suggesting that H$_2$O$_2$ and the OH$^-$ radical but not super oxide is involved. ROS treatment of endothelial cells induce the focal adhesion kinase pp 125 PAK, a cytosolic tyrosine kinase which has been implicated in the oxidant-mediated adhesion process. [101]

This section and the section (A). Ageing are closely related as ROS and RNS are widely implicated in the inflammatory and ageing process. [102]

Recently, Syad MA, Pietropaolo M, and colleagues [103] published a paper entitled: Is type 2 diabetes a chronic inflammatory / autoimmune disease? They were able to detect a subset of patients with T2DM in which an acute phase response seemed to be associated with islet cell autoimmunity. They were able to demonstrate that 12 % of patients age 65 and older had islet cell autoantibodies (ICA) and GAD. They also were able to detect a significant increase in fibrinogen (P= 0.005) and C-reactive protein levels (P= 0.025) in patients with high levels of GAD 65 and/or IA-2 autoantibodies as compared with antibody negative patients and control subjects. [104]

This group of T2DM has been referred to by Zimmet and others as latent autoimmune diabetes mellitus in adults (LADA) [105]

This information points to the presence of the acquired immune (humoral islet cell autoimmunity) system being in play in a subset of older as well as younger patients with T2DM and that this system is significantly associated with the downstream acute phase reactants of the innate immune system: C-reactive protein and fibrinogen. This same delicate interplay of the two immune systems may well be in play in the development of atheroscleropathy as we know there are autoantibodies to oxidized LDL-C. As further knowledge emerges regarding these two immune systems and how they interact we may have an even better understanding of the complex mechanism of MS, IR, PD, T2DM, and atheroscleropathy.

The current medical literature has provided us with a growing body of knowledge in studies of basic science, epidemiology, animal, and even human clinical trials that implicate inflammation in the pathogenesis of MS, IR, PD, T2DM and atheroscleropathy with their morbid, deadly intersection.

The above is information is incomplete and just a small portion of information was presented to set the stage regarding the role of inflammation in the intima and islet. In summary, there are two common threads that weave these two diseases (T2DM and accelerated atherosclerosis) together, resulting in the complex mosaic fabric of atherosclerosis:

Redox stress and inflammation. (figure 6)
It is interesting to note that both HMG CoA Reductase inhibitors (statins) and ACE inhibitors and ARBs are having such a profound effect on non diabetic atherosclerosis and hypertension and an equal if not greater reduction in
events in the treatment of diabetic associated hypertension, atheroscleropathy, and even delaying or preventing the development of overt T2DM. (table 9) Note that the three drug classes all have a direct or indirect positive effect on inflammation and redox stress. Utilizing the RAAS acronym may have a positive effect on event outcomes at the morbid mortal intersection associated with the interweaving threads of redox stress and inflammation which result in atheroscleropathy.

(G). Glucotoxicity
Glucotoxicity is associated with both type 1 and type 2 diabetes mellitus and, thus, the similarly shared multipleopathies associated strongly with redox stress (figure 7). There is a major difference between T1DM and T2DM in regards to atheroscleropathy. In T1DM the atheroscleropathy does not start until there is a diagnosis and glucotoxicity develops acutely. In contrast T2DM is preceded by 5–10 years of a MS, IR, and PD state consisting initially of postprandial glucose elevations (IGT) then fasting glucose elevation (IFG). Additionally, MS and IR comes with the attendant A-FLIGHT toxicities. (table 3)

Four subsections are important in this discussion.

I. AGEs were discussed in section (A)
II. Autoxidative reactions
Autoxidative reactions occur as monosaccharides, and fructose-lysine can spontaneously reduce molecular oxygen.

Figure 7
Glucotoxicity and intimal redox stress injury to the arterial vessel wall in atheroscleropathy initially associated with glucose elevations post prandial then fasting as in prediabetes (PD). Stages III and IV. Transitioning to overt T2DM on the continuum. (table 8)
The reduced oxygen products formed are $\text{O}_2^-$, OH$^-$, and $\text{H}_2\text{O}_2$. Each of these ROS can contribute to damaging lipids and proteins through cross-linking and fragmentation. [106–108]

The process of combined autooxidation and glycation is frequently referred to as glycoxidation which is another common process of protein modification. The ROS from these reactions serve not only as the source for autoxidation but also fuel the cycle of AGE formation (ROS beget ROS).

Autooxidation occurs at the site of the protein component embedded within the LDL cholesterol particle resulting in glycated LDL and glycoxidated LDL cholesterol which contribute to its retention just as oxidized LDL is retained within the intima which initiates and sustains atherogenesis and subsequent atheroscleropathy.

Native LDL is not atherogenic and is not retained within the intima; however, if it becomes modified by oxidation, glycation, glycoxidation or homocysteinated, it becomes modified and retained (trapped to adjacent glycosaminoglycans and structural glycoproteins) to initiate, maintain, and accelerate the atherogenic process within the intima.

III. The Polyol – sorbitol pathway

The polyol – sorbitol pathway is also driven by an excess production of glucose. Glucose is converted to sorbitol by aldose reductase at the expense of NADH/NAD(P)H being converted to NAD$^+/\text{NAD}(P)^+$. Sorbitol is then converted to fructose by sorbitol dehydrogenase at the expense of NAD$^+$ NAD(P)$^+$ being converted to NAD$^+/\text{NAD}(P)^+$. [60–62]

This reductive stress (pseudohypoxia) of the polyol – sorbitol pathway thus amplifies the redox stress within the islet milieu. This singular pathway is of great importance as it is the major pathway for supplying unpaired unstable electrons through the process of reduction. This reductive stress is dependent upon hyperglycemia associated with both T1DM and T2DM. Postprandial hyperglycemia results in reductive stress even before overt T2DM has developed.

Were it not for the importance of this singular source of reductive stress, this review could have been entitled “Intimal Oxidative Stress”.

IV. Glucose scavenging of nitric oxide

Endothelial dysfunction is strongly associated with both T1DM and T2DM. Brodsky et al. have recently been able to demonstrate that glucose is capable of directly scavenging NO resulting in the chemical inactivation of NO. They were able to conclude that the glucose-mediated NO loss may directly contribute to hypertension and endothelial dysfunction in diabetic patients. [109]

The authors were also able to show a glucose-mediated decline in the lifetime of NO. These findings may have a direct, deleterious effect of decreasing the naturally occurring antioxidant capability of NO.

Glucotoxicity increases oxidative stress as demonstrated by increased 8-hydroxy-2'-deoxyguanosine (8-OhdG, a marker for oxidative stress) found in the urine and mononuclear cells from blood in T2DM patients.

Ihara et al. found higher levels of 8-OhdG and 4-hydroxy-2-nonenal- (HNE)-modified proteins in pancreatic beta-cells of GK rats (a model of non-obese type 2 diabetes) than in control Wistar rats. These levels increased proportionally with age. [110]

Section "(F). Free Fatty Acids" would lead one to believe that a lipocentric view is of extreme importance and may be playing the dominant role in beta cell dysfunction and insulin resistance.

Poitout and Robertson [111] have recently pointed out (with strong supporting data) that glucotoxicity is a prerequisite for lipotoxicity. They propose that chronic hyperglycemia (independent of hyperlipidemia) is toxic for beta-cell function, whereas chronic hyperlipidemia is deleterious only in the context of concomitant hyperglycemia. With time, both glucotoxicity and lipotoxicity contribute to the progressive deterioration of glucose homeostasis and beta cell dysfunction. Seldom do either of these two toxicities exist alone in the postprandial clinical setting of MS, IR, PD, and T2DM, and both contribute to the excess redox stress associated with the other A-FIGHT toxicities, having an overall multiplicative effect within the intima and islet. [111]

Before leaving this section on glucotoxicity it is important to note that cytosolic production of superoxide [O2$^-$] results in the activation of protein kinase C, increased formation of glucose-derived advanced glycation products, and an increased flux through the polyol – sorbitol pathway. Nishikawa T et al. was able to show nicely that by blocking mitochondrial derived O2' with manganese superoxide dismutase or uncoupling mitochondrial oxidative phosphorylation they were able to prevent the above cytosolic perturbations. [112] Additionally, Pennathur S et al. were able to demonstrate in the Cynomologus monkey that streptozotocin induced diabetes resulted in a hydroxyl radical-like species which oxidized artery wall proteins. The oxidative products, ortho-tyrosine and meta-tyrosine correlated strongly with serum levels of glycated...
hemoglobin. In these early lesions 3-nitrotyrosine was not correlated to the glycated hemoglobin. [113]

(H). Hypertension toxicity
Hypertension is associated with increased redox stress and ROS activity. Furthermore, hypertension is associated with ROS mediated vascular damage and is closely associated with the activation of Ang II (see section (A). Angiotensin II) and its effect on the vascular NAD(P)H oxidase superoxide (O₂⁻) generating enzyme. [26]

Cellular sources of vascular superoxide production are the endothelial cell, vascular smooth muscle cell and adventitial fibroblasts. The major enzymatic sources are NAD(P)H oxidase, xanthine oxidase and, paradoxically, the eNOS enzyme (in the presence of oxidative stress or deficiency of L-arginine or tetrahydrobiopterin). [114]

It is important to note that glucotoxicity is closely associated with activation of the RAAS at the local, interstitial and tissue levels.

Recently, amylin has been implicated as being elevated in patients who have a positive family history associated with hypertension and is elevated prior to the onset of hypertension when insulin remains at the normal level. Thus, amylin levels may become a screening tool for the development of future essential hypertension. [115]

Hypertension is associated with the clustering phenomenon of the metabolic syndrome and its importance to the overall picture of redox stress is not to be underestimated as it contributes significantly to the overall morbidity and mortality associated with T2DM. [116,117]

(H). Homocysteine toxicity
The general population of diabetics (T1DM and T2DM) will, in all probability, have the same amount of gene polymorphism of the folate-dependent methylene tetrahydrofolate reductase gene with subsequent mild to moderate hyperhomocysteinemia (hHcy) which occurs in 10–15% of the general population. [118–120]

This gene polymorphism is especially important in those individuals with a decrease in dietary folate. hHcy can be improved with folate supplementation and can improve endothelial-dependent endothelial cell dysfunction. [121]

Homocysteine (Hcy) is not usually elevated as a direct result of diabetes 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. [122]

This plays an extremely important role for those diabetic patients on dialysis. [123] 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 thioli and reducing glutathione pools and increasing GPx activity with restoration of the endothelial function. [124]

The ensuing cellular redox stress is magnified and total Hcy consumes NO by the indirect process of O₂⁻ converting NO to toxic peroxynitrite (ONOO⁻).

In addition to ONOO⁻ formation, NO in conjunction with thiols and oxygen radicals generate nitrotyrosine and nitroarginine which compete for the substrate eNOS in a feedback mechanism, limiting further NO generation. [125–127] As a result, there is endothelial cell dysfunction, endothelial cell toxicity and endothelial cell loss, increased ROS, increased ONOO⁻ and decreased NO associated with hHcy. [128] hHcy is multiplicative in nature and even though its effects may occur later in T2DM than the other associated toxicities, it has a devastating effect on endothelial cell function.

Presently, we know there are other toxicities operating within the renal glomerulus producing microalbuminuria (reflecting endothelial cell dysfunction and damage) at a stage prior to the declining glomerular filtration rate responsible for hHcy.

A recent clinical study by Maejima et al. [129] revealed significant elevated levels of ONOO⁻ peroxynitrite (by Griess method) in 126 T2DM patients as compared to 76 non-diabetic controls. 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 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. [130] were able to demonstrate that increased concentrations of Hcy resulted in a decreased NO response to bradykinin and L-arginine.

They were able to show that Hcy stimulated the formation of superoxide anions and peroxynitrite with increased levels of nitrotyrosine. The addition of 5-methyltetrahydrofolate restored NO responses to bradykinin and L-arginine.
Table 8: Courtesy [8,9,53] The five stages of T2DM: the natural progressive history of T2DM

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
</table>
| I. LATENT STAGE:[EARLY] | Insulin Resistance:  
- Genetic Component  
Beta Cell Defect: (Dysfunction)  
- Genetic  
- Intracellular/extracellular amylin fibril toxicity. Abnormal processing, storage or secretion.  
Intra-Islet Endothelial Absorptive Defect:  
- Heparan sulfate proteoglycan (HSPG) PERLECAN of the capillary endothelial cells avidly attracts amylin (IAPP) and the islet amyloid forms an envelope around the capillary. This is in addition to the increase in the basement membrane associated with the pseudohypoxia (associated with glucotoxicity) and the redox stress within the capillary. |
| II. TRANSITION STAGE:[MIDDLE] | Persistent Hyperinsulinemia  
Persistent Hyperamylanemia  
- Continued remodeling of the endocrine pancreas (amyloid).  
- Beta cell displacement, dysfunction, mass reduction and diffusion barrier. |
| III. IGT STAGE (Impaired Glucose Tolerance):[LATE] | "Pre-diabetes" Human Health Services (HHS) and American Diabetes Association (ADA) term.  
[Start treatment at this time]  
[Diagnose earlier: rejuvenation of the 2 hour glucose tolerance blood sugar 140–199 mg/dL]  
| IV. IFG STAGE (Impaired Fasting Glucose):[LATER] | "Pre-diabetes" Human Health Services (HHS) and American Diabetes Association (ADA) term.  
[Blood sugar ranging 110–126 mg/dL]  
[Impaired hepatic glucose production]  
- Increasing global insulin resistance (hepatic) with subsequent gluconeogenesis. Feeding forward in the vicious cycle to accelerate insulin resistance globally. |
| V. OVERT STAGE:[TO LATE] | [50% loss of beta cell function]  
Ya, Vb, Vc: Phases I, II, III: mild, moderate/severe, complete. Use medications that do not increase insulin or amylin. Use combination therapy. Start treatment at stage III-IV (IGT-IFG).  
- Paradigm Shift. Start treatment at the earlier stage of IGT. |

Table 9: Courtesy [8,9,53] The RAAS acronym: for the prevention and treatment of redox stress in atheroscleropathy and stabilization of the vulnerable intima and islet in T2DM

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>Reductase inhibitors (HMG-CoA). Decreasing modified LDL cholesterol, i.e. oxidized, acetylated LDL cholesterol. Improving endothelial cell dysfunction. Thus, decreasing the oxidative stress to the arterial vessel wall and the islet. Redox stress reduction.</td>
</tr>
</tbody>
</table>
| A | 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 A-FLIGHT toxicities. Plus the direct/indirect antioxidant effect within the islet and the arterial vessel wall. Redox stress reduction.  
Aspirin antiplatelet, anti-inflammatory effect. |
| A | Aggressive control of diabetes. Decreasing modified LDL cholesterol, i.e. glycated LDL cholesterol. Improving endothelial cell dysfunction. Also decreasing glucose toxicity and the redox stress to the intima and pancreatic islet. Aggressive control of Hcy with folic acid with its associated additional positive effect on re-coupling of the BH4 cofactor with the eNOS reaction to produce eNO. Redox stress reduction. |
| S | Statins. Improving plaque stability (pleiotropic effects) independent of cholesterol lowering. Improving endothelial cell dysfunction and preventing the angiogenesis associated with arterial vascular remodeling which destabilizes the unstable atherosclerotic plaque. Plus, the direct/indirect antioxidant anti-inflammatory effects within the islet and the arterial vessel wall promoting stabilization of the unstable, vulnerable islet and the arterial vessel wall. Style: Lifestyle modification: Stop smoking, lose weight, exercise, and change eating habits. Redox stress reduction. |
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 μM 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" (Table 7) were able to increase NO as well as decreasing cellular cholesterol. [131]

Stuhlinger MC 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 inhibit nitric oxide synthesis. 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. [132]

(T). Triglyceride toxicity
Multiple lipases (intestinal, muscular – both skeletal and cardiac-, adipose, and hepatic) are responsible for the dynamic flux between the long chain fatty acids (LCACoA esters) and the glycerol molecular backbone of the triglycerides (see section (F). Free Fatty Acids”).

Hypertriglyceridemia certainly plays a role in toxicity regarding the development of redox stress, not only its role in lipotoxicity and FFA toxicity discussed previously, but independently as its own marker of toxicity. There is a close association of hypertriglyceridemia and the atherogenic small dense LDL cholesterol particles which are more likely to be oxidized and contribute to redox stress. This condition is central to the lipid triad [133].

We need to recall that Apo E is responsible for carrying this lipid fraction and that the Apo E -/- knockout mouse develops atherosclerosis at an accelerated rate. We need to also bear in mind that gene polymorphism may play a role in the development of ADIA since Apo E is an important part of all amyloid formation and stabilization. Kahn et al. were able to demonstrate in the human islet amyloid polypeptide transgenic mouse model that these mice did not develop islet amyloid unless fed a high fat diet [134].

Stored neutral triglycerides provide the substrate for FFA production which can be immobilized immediately by exercise or stress induced lipolysis.

When these are stored in ectopic non-adipose cells such as the cardiac and skeletal myocyte, the endothelial cell or the islet beta cell, they are capable of causing cellular dysfunction or lipoapoptosis as discussed in the sections "(F). Free fatty acids" and "(L). Lipotoxicity – Specific".

As stated earlier in this paper, it is difficult to separate these three moieties as they are closely interconnected with each other and within the manifold A-FLIGHT toxicities.

Redox stress and matrix metalloproteinases
Elevated redox stress is associated with an increase in matrix metalloproteinase (MMP) activity, especially the inducible MMP-9 [135].

This would be associated with an increase in extracellular matrix (ECM) remodeling and would contribute to increased intima media thickness within the arterial vessel wall. This would accelerate the process underlying the similar mechanism of AGEs with a stiffening and decreased compliance of the arterial vessel wall which would contribute to diastolic dysfunction of the arterial vessel wall. The acceleration of the stiffness of the arterial vessel wall would contribute to hypertension, specifically systolic hypertension. This mechanism may be in play in the clustering of hypertension in the metabolic syndrome.

Cells are dependent on integrin matrix ligand binding sites and MMP-9 is a basement membrane degrading enzyme. Cells are constantly re-establishing new integrin matrix binding sites. However, if there is a complete disconnection of integrin matrix binding sites due to a robust increase in MMP-9, the cell may undergo apoptosis. MMP-9 was recently shown to be elevated in diabetes mellitus and, in addition, the role of redox stress was shown to play an important role [135].

A robust activation of MMP-9 may result in a complete disconnection of the beta cell and the surrounding ECM with resultant apoptosis. Recently, in our laboratory, we have been able to demonstrate decreased endothelial cell density with increased apoptosis of endothelial cells in the hearts of mice treated with alloxan vs. controls.

We were also able to show a decrease in NO and an increase in peroxynitrite and ROS in these same animals thus, linking the importance of cellular apoptosis, MMP-9 and redox stress. We then compared these findings of alloxan-induced diabetes in MMP-9 knockout mice to alloxan-induced diabetes in the wild type. Alloxan-induced
diabetes MMP-9 -/- mice did not have induced apoptosis and did not have a decrease in endothelial cell density when compared to wild type alloxan-induced diabetes (unpublished data).

These findings may apply to the beta cell within the islet, as all cells require an integrin matrix binding for survival. The MMP-9 may also decrease the larger size amylin derived islet amyloid fibrils to the more intermediate size toxic amyloid particles and contribute to apoptosis as described by Janson et al.[136] (Table 5).

MMP-9 has also been shown to be elevated in laminitic horses having digestion of the basement membranes with resultant separation of the epidermal and dernal lamina. [137] These same processes within the islet could be responsible for a loss of intracapillary endothelial cells which would decrease the rate at which they could pick up newly synthesized insulin and transport it to the systemic circulation, and provide a mechanism for the delay in first phase insulin secretion which is typical of T2DM and even impaired glucose tolerance.

MMP-9 may even play a role in the clearing of ECM in order to allow for the space-occupying lesion of ADIA deposition. Redox stress (signaling) activates MMPs (Table 5).

Conclusion
Throughout this review, we have tried to remain focused on the relationship between redox stress and ROS in the intima and at times the islet, and how these two interact with the multiplicative effect of the A-FLIGHT toxicities of MS, IR, PD, and overt T2DM to induce atheroscleropathy. The reader will note that redox stress and ROS operate through similar mechanisms and will operate in a similar fashion in other chronic disease states such as chronic inflammatory diseases (pancreatitis, rheumatoid arthritis, ulcerative colitis, Crohn’s), ageing, cancer, ischemia/ischemia-reperfusion injury, hypertension, diastolic/systolic dysfunction, congestive heart failure, diabetic and nondiabetic nephropathy and neurodegenerative diseases.

When redox homeostasis transitions to redox stress, redox signaling ensues in all tissues and organs regardless of the multiple similar or dissimilar etiologies.

Redox stress is a redox signaling system. [101,138]

Reflections and future directions
An article entitled “Diabetes as a manifold disease” (previously published in the February 8th, 1902 issue of JAMA) was reprinted in the February 13th, 2002 issue of JAMA, in the section on 100 years ago. [139,140]

T2DM and associated atheroscleropathy remain a heterogeneous and manifold disease not only in their etiology but also in their manifold toxicities associated with MS, IR, PD, and T2DM.

In 1902, the author (unknown) could not have envisioned the exponential growth of T2DM and atherosclerosis we are currently experiencing.

Just as this author pointed to a new concept, we have attempted, in this review, to outline the important contemporary concept of intimal redox stress and the rusting – rancid intima within vulnerable atherosclerotic plaques.

Treatment and potential prevention of these diseases can be accomplished through global risk reduction of the manifold toxicities by using the currently available treatment modalities we now have at our disposal. [141]

If the current trend continues (due to the current epidemic of our adolescent youth and the aging baby boom generation) these patients are going to be seen in our offices with increasing frequency.

Recently, Dagogo-Jack S discussed primary prevention of diabetes, secondary prevention (of diabetic complications), and tertiary prevention (of morbidity and mortality from established diabetic complications) utilizing multiple drug targets in the management of T2DM.

Currently, a focus on global risk reduction is necessary for a more complete approach by physicians providing care for the diabetic patient.[142,143] By aggressively reducing the elevated substrates producing the A-FLIGHT toxicities and ROS (table 3) and using the simple RAAS acronym (table 9) we may be able to restore our individual, endogenous, potent, antioxidant network. A better understanding of redox stress and redox homeostasis will enable each of us to develop a shift in the treatment paradigm for the quartet of MS, IR, PD, T2DM, and the associated cardiovascular atheroscleropathy due to the loss of metabolic and redox homeostasis.

Abbreviations
8-OhdG: 8-hydroxy-2′-deoxyguanosine; ADA American Diabetes Association; ADIA: amylin derived islet amyloid; AFEs: advanced fructosylation endproducts; AGEs: advanced glycation endproducts; Ang II: angiotensin II; AT-1: angiotensin type 1; ACEi: angiotensin converting enzyme inhibitors; ARBS: angiotensin II receptor blockers; eNO: endothelial nitric oxide; eNOS: endothelial nitric oxide synthase; FFA: free fatty acid; GPx: glutathione peroxidase; GSH: glutathione reduced form; GSSG: glutathione disulfide; Hcy: homocysteine; HNE: 4-hydroxy-2- nonenal; IAPP: islet amyloid polypeptide; iNO: inducible
nitrergic oxide; iNOS: inducible nitric oxide synthase; ISTAPs: intermediate sized toxic amyloid particles; LCA-
CoA: long chain acyl-Coenzyme A; Nfkappa B: nuclear factor kappa B; nNOS: neural nitric oxide synthase; NO:
nitric oxide; NOS: nitric oxide synthase; O2-: superoxide; OH-: hydroxyl radical; ONOO-: peroxynitrite; RAGE: re-
ceptor for advanced glycation endproducts; SOD: superoxide dismutase; T1DM: type 1 diabetes mellitus; T2DM:
type 2 diabetes mellitus.

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