# Islet Redox Stress: The Manifold Toxicities of Insulin Resistance, Metabolic Syndrome and Amylin Derived Islet Amyloid in Type 2 Diabetes Mellitus

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## ABSTRACT

**Context** Redox stress, reactive oxygen species, reactive nitrogen species, and oxygen free radicals ("toxic oxygen") are increasingly being reported as important cellular signaling mechanisms. It has been known for over a hundred years that type 2 diabetes mellitus is a manifold disease, not only in its etiology, but also in its associated manifold toxicities and multiple complications of the diabetic opathies. The presence of islet amyloid has also been described in association with type 2 diabetes mellitus for a century.

**Objective** This review will attempt to remain focused on the relationship between redox stress, the reactive oxygen species and the reactive nitrogen species in the islet, and how these interact with the multiplicative effect of the toxicities of insulin resistance, metabolic syndrome, amylin (hyperamylinemia), amylin derived islet amyloid and type 2 diabetes mellitus.

**Conclusions** Redox sensitive cellular signaling systems play an important role in the development, progressive nature (remodeling) and damaging effects on the beta cell within the islet of the pancreas. Furthermore, redox stress may play an important role in the remodeling and development of islet amyloid creating a space-occupying lesion with a resultant secretory and absorptive defect within the islet.

The presence of manifold toxicities necessitates an approach of global risk reduction in the prevention and treatment of type 2 diabetes mellitus. An improved understanding of the dynamic relationship between these toxicities and redox stress within the islet will aid both the researcher and the clinician.

## BACKGROUND

Transference of electrons between oxygen species (cellular respiration) allows each of us to survive on this planet, not only at the cellular level but also as an organism. Redox cycling describes the normal physiologic process of reduction and oxidation in order to pair anew unstable, damaging, reduced reactive oxygen species (ROS) which is meant to include the oxygen free radicals ($O_2^-$: superoxide; $H_2O_2$: hydrogen peroxide; $OH^-$: hydroxyl radical; singlet oxygen) and organic analogues which would include the reactive nitrogen species (RNS) which is primarily peroxynitrite ($ONOO^-$). Redox cycling thus implies a homeostatic balance between ROS production and antioxidant capacity, and is also termed redox homeostasis. In contrast, redox stress (redox imbalance) implies a loss of this unique homeostasis with an excess production of ROS (Tables 1 and 2) either through the process of reduction or that of oxidation. Oxidative stress implies a loss of homeostasis

**Table 1**. Origins of reactive oxygen species (ROS) which produce redox stress.

| | |
|---|---|
| **I** | Excess $O_2$ (oxygen therapy) |
| **II** | Absorption of radiant energy (ultraviolet light) or ionizing radiation (radiotherapy) |
| **III** | Exposure to toxins: carbon tetrachloride, dioxin, alloxan and streptozotocin to name just a few |
| **IV** | Reduction-oxidation (redox) reactions during normal physiologic processes (cellular respiration) |

A. Respiratory chain enzymes and oxygen
B. Xanthine oxidase
C. Cytochrome P450 monooxygenase activity
D. NAD(P)H / NADH oxidase
E. Fenton reaction: $Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH + OH^-$
F. Haber-Weiss Reaction $H_2O_2 + O_2^- \rightarrow \underline{-OH^-} + O_2 + OH^-$

| | |
|---|---|
| **V** | Ischemia - Ischemia reperfusion injury |
| **VI** | Inflammatory processes. Acute and chronic |
| **VII** | Once free ROS radicals form, they can react with membrane lipids, proteins and nucleic acid to initiate auto-catalytic reactions (ROS beget ROS) |

with an excess of ROS by the singular process of oxidation.

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 non-flexible aged proteins, specifically collagen of the extracellular matrix) [1].

The evolutionary process of redox cycling allows humans to survive in an atmosphere of high oxygen content. In addition, our bodies have become accustomed to utilizing the

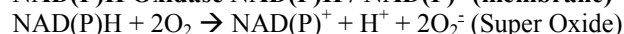**Table 2.** Origins of reactive oxygen species (ROS) and cellular location.

**Nicotinamide adenine dinucleotide reduced (NADH)**
**NADH Oxidase NADH / NAD$^+$ (mitochondrion, cytosol)**
$NADH + 2O_2 \rightarrow NAD^+ + H^+ + \underline{2O_2^-}$ (Super Oxide)
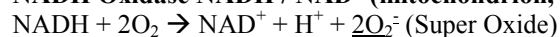
**Nicotinamide adenine dinucleotide phosphate reduced (NAD(P)H)**
**NAD(P)H Oxidase NAD(P)H / NAD(P)$^+$ (membrane)**
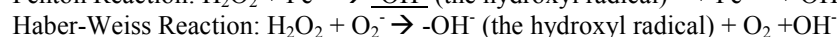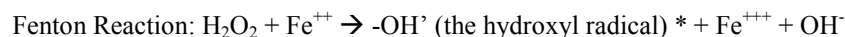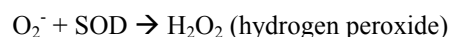$NAD(P)H + 2O_2 \rightarrow NAD(P)^+ + H^+ + \underline{2O_2^-}$ (Super Oxide)

**Super oxide dismutase (SOD):**
MnSOD = Mitochondrial SOD
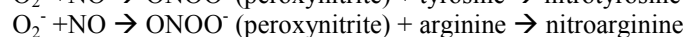CuZnSOD = Intracellular (cytosolic) SOD
EcSOD = Extracellular SOD

$O_2^- + SOD \rightarrow H_2O_2$ (hydrogen peroxide)

Fenton Reaction: $H_2O_2 + Fe^{++} \rightarrow \underline{-OH'}$ (the hydroxyl radical) * $+ Fe^{+++} + OH^-$
Haber-Weiss Reaction: $H_2O_2 + O_2^- \rightarrow \underline{-OH^-}$ (the hydroxyl radical) $+ O_2 + OH^-$

**Peroxynitrite: origins of reactive nitrogen species (RNS)**
$O_2^-$ is consumed. Nitric oxide (NO) is also consumed in this process with the creation of reactive nitrogen species (RNS).
$O_2^- + NO \rightarrow ONOO^-$ (peroxynitrite) + tyrosine $\rightarrow$ nitrotyrosine
$O_2^- + NO \rightarrow ONOO^-$ (peroxynitrite) + arginine $\rightarrow$ nitroarginine
Nitroarginine competes for arginine in the formation of eNO.
Nitrotyrosine reflects redox stress and leaves an indelible measurable footprint.
NO: the good; $O_2^-$: the bad; $ONOO^-$: the ugly [81]
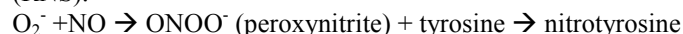
**Table 3.** The manifold toxicities of insulin resistance, metabolic syndrome and T2DM.

| | A-FLIGHT toxicities | |
|---|---|---|
| **A** | Amylin (hyperamylinemia)/amyloid toxicity | ROS |
| | Ang II (also induces PKC) | ROS plus PKC |
| | AGEs/AFEs (advanced glycosylation/fructosylation endproducts) | ROS |
| | Antioxidant reserve compromised | ROS |
| | Absence of antioxidant network | ROS |
| | Ageing | ROS |
| **F** | Free fatty acid toxicity | ROS |
| **L** | Lipotoxicity | ROS |
| **I** | Insulin toxicity (hyperinsulinemia-hyperproinsulinemia) (endogenous) | ROS |
| | Inflammation toxicity | ROS |
| **G** | Glucotoxicity (compounds peripheral insulin resistance) reductive stress | ROS plus PKC |
| | Sorbitol / polyol pathway | |
| | Pseudohypoxia (NADH/NAD increased) | |
| **H** | Hypertension toxicity | ROS |
| | t homocysteine toxicity | ROS |
| **T** | Triglyceride toxicity | ROS |

See reference [2]

mechanism of redox stress injury to fend off invading infectious organisms in order to survive our environment. Paradoxically (when there is a loss of homeostasis resulting in redox or oxidative stress), this protective mechanism acts on our own cells and causes damage to multiple organs, especially the islet in type 2 diabetes mellitus (T2DM).

T2DM is now considered to be a global epidemic and we need to re-evaluate the natural history of this disease and include the histological presence of the islet amyloid and its association with redox stress in the islet.

In patients with insulin resistance, metabolic syndrome and T2DM, there is an elevated tension of redox stress within the islets of the pancreas due to multiple toxicities (Table 3). Each of the A-FLIGHT toxicities results in the formation of damaging ROS [2].

The beta cell is unique in that it is unusually susceptible to the damaging role of ROS and this may be why alloxan and streptozotocin induce selective destruction of the beta cell with ensuing type 1 diabetes mellitus (T1DM) in various animal models. Alloxan has been shown to induce $O_2^-$ and streptozotocin inducible nitric oxide (iNO) which results in the selective destruction of the beta cells within the islets [3, 4]. This susceptibility makes the beta cell a unique target and vulnerable for selective destruction by the ROS associated with the A-FLIGHT toxicities

(Table 3). Not only are ROS involved in the development of T1DM and T2DM but they also play an important role in the long-term development of associated complications [5, 6].

## INSULIN RESISTANCE AND THE METABOLIC SYNDROME

Insulin resistance describes the condition whereby there is a resistance to insulin-mediated glucose uptake by some cells. Insulin resistance is central to the clustering of multiple metabolic abnormalities and clinical syndromes (Figure 1). There are many names for the clustering of multiple metabolic abnormalities and clinical syndromes (Table 4). In 1999, the World Health Organization

**Table 4.** The myriad names of the metabolic syndrome

1. The insulin resistance syndrome
2. Syndrome X
3. Reaven syndrome
4. Metabolic syndrome (preferred term by WHO)
5. Metabolic syndrome X
6. Multiple metabolic syndrome
7. Plurimetabolic syndrome
8. Dysmetabolic syndrome
9. Cardiovascular dysmetabolic syndrome
10. Cardiometabolic syndrome
11. The "H" phenomenon
12. The "Deadly quartet"

chose a unifying definition for this syndrome and elected to adopt the term "metabolic syndrome" [7]. In the USA, metabolic syndrome was given special consideration by the National Cholesterol Education Panel - Adult Treatment Panel Three Guidelines (NCEP-ATP III) 2001.

In addition, data from the third report of the National Health and Nutrition Examination Survey (NHANES III) indicate that 47 million people in the USA suffer from this condition using census data from the year 2000 [8, 9]. It is appropriate that insulin resistance and metabolic syndrome be recognized as being very important for cardiovascular disease and the development of overt T2DM due to its multiplicative effect of risk factors and the underlying causes of an elevated tension of redox stress and ROS.

Insulin resistance and the metabolic syndrome are associated with multiple toxicities and each of the A-FLIGHT toxicities are associated with an increase in ROS which create an elevated tension of redox stress within the islet, contributing to an unstable milieu.

As nature attempts to pair anew these unpaired unstable electrons, there will be a considerable amount of damage to the vulnerable beta cell, amylin and the islet (Table 3, Figures 2 and 3).



**Figure 1.** Metabolic syndrome: insulin resistance is central to the clustering phenomenon of hypertension, hyperinsulinemia, hyperamylinemia, hyperlipidemia (lipid triad and visceral obesity), hyperglycemia (overt T2DM), hyperuricemia, elevation of PAI-1, polycystic ovary syndrome, microalbuminuria and the increased incidence of coronary heart disease.
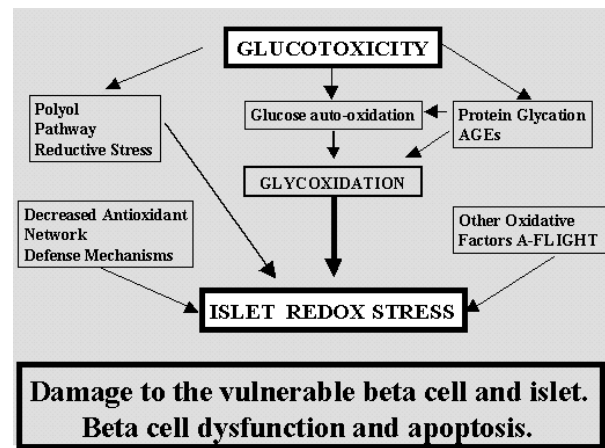


**Figure 2.** Glucotoxicity and islet redox stress: glucotoxicity contributes to islet redox stress through the polyol sorbitol pathway of reductive stress, the glucose auto-oxidation process, protein glycation and AGE formation, glycoxidation, decreased antioxidant network defense mechanisms and other oxidative factors of A-FLIGHT toxicities all contribute to the damaging effects to the vulnerable beta cell and islet.

## A-FLIGHT TOXICITIES (Tables 3 and 5)

### (A). Angiotensin II

Angiotensin II (Ang II) is associated with hypertension 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 islet as angiotensin type one (AT-1) receptors have been identified as being present on both the endothelial and the beta cells within the islet [10, 11]. Insulin is known to upregulate the AT-1 receptor [12] and there exists cross talk between the insulin and the Ang II signaling systems [13]. In 1995, Copper *et al.* were able to demonstrate that amylin activates the RAAS with elevations in renin and aldosterone in humans [14] 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 [15]. Taken together, these data support the strong influence of a local RAAS mechanism operating within the islet for the local production of excess Ang II. The islet is quite vascular with an abundant supply of intra islet capillaries and endothelial cells and,
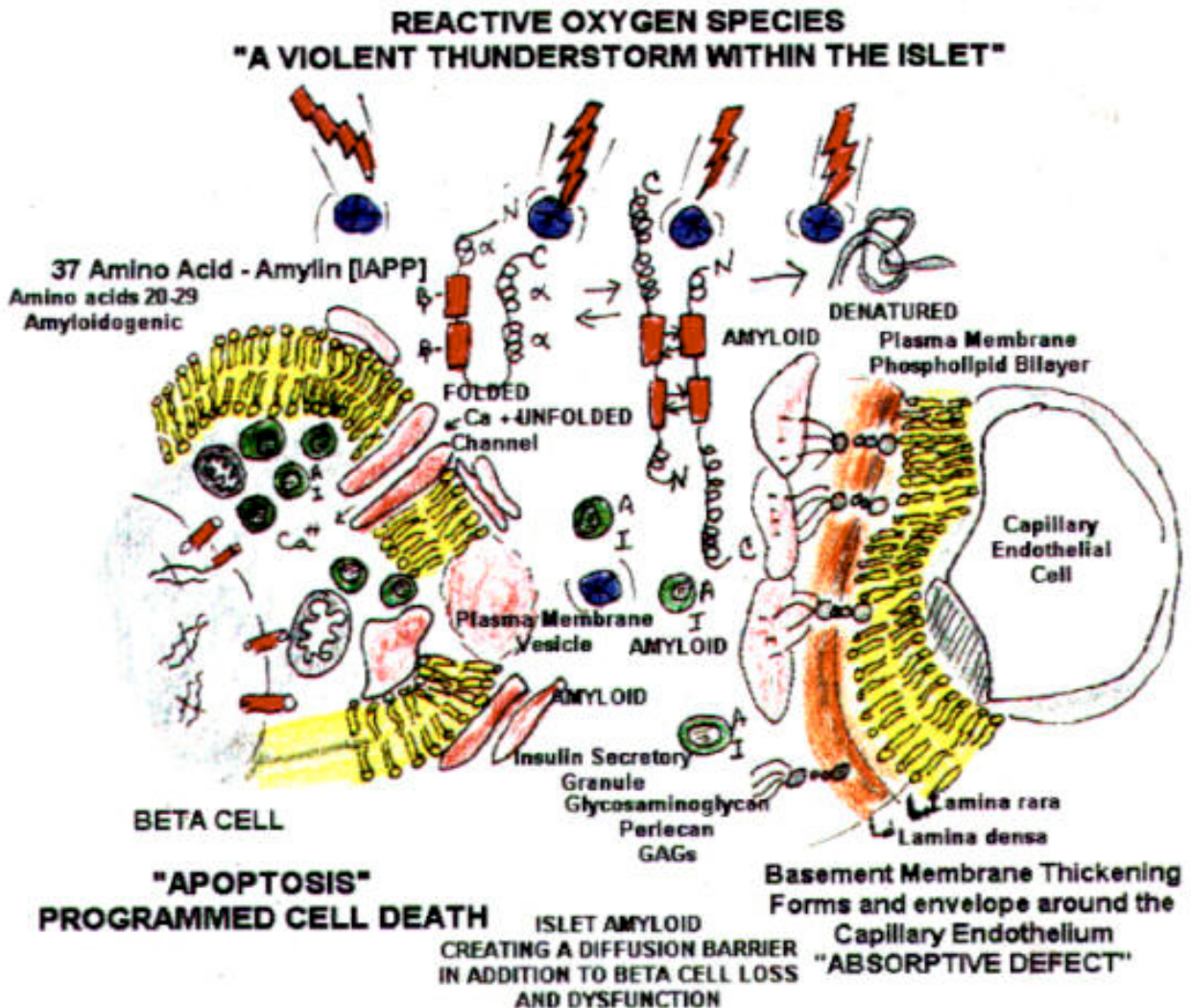
**Figure 3.** Reactive oxygen species creating a violent situation within the islet. Superoxide, the hydroxyl radial and hydrogen peroxide all are capable of causing damaging effects within the islet and the vulnerable beta cell which may cause an unfolding of the native secondary structure of amylin allowing this amyloidogenic structure to refold (misfold) into the antiparallel crossed beta sheet structure of islet amyloid creating a secretory and absorptive defect within the islet as well as a space occupying lesion.

therefore, the vascular NAD(P)H oxidase enzyme comes into play. Ang II is one of the most potent endogenous stimula for the generation of superoxide $O_2^-$ via the activation of vascular NAD(P)H oxidase [16, 17]. 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 [18].

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) [19]. 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 insulin resistance, metabolic syndrome and T2DM.

In the insulin resistance state and metabolic syndrome, the 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 vulnerable islets.

## (A). Advanced Glycosylation Endproducts

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

**Table 5.** The manifold toxicities: ROS. Cytotoxicities, apoptosis and lipoapoptosis.

| | | |
|---|---|---|
| **1.** | ISTAPs (intermediate sized toxic amyloid particles). ROS | <u>Amylin (IAPP)/amyloid toxicity</u><br>[Cell membrane damage]<br>[Cytotoxicity of the beta cell]<br>{Apoptosis} |
| **2.** | Free fatty acid: LCACoA (long chain acyl Co enzyme A). Products of lipid peroxidation. | <u>Lipotoxicity</u>. ROS {Lipoapoptosis} |
| **3.** | Ceramide: amino alcohol with LCACoA. Attached to the amino group. | <u>Lipotoxicity</u>. ROS {Lipoapoptosis} |
| **4.** | Triglycerides. | <u>Lipotoxicity</u>. ROS |
| **5.** | Glycation. ROS | <u>Glucotoxicity</u>. Protein alteration |
| **6.** | Glycoxidation. ROS | <u>Glucotoxicity</u>. Protein alteration |
| **7.** | Autoxidation. ROS | <u>Glucotoxicity</u>. Protein alteration |
| **8.** | Advanced glycation endproducts (AGEs). ROS | <u>Glucotoxicity</u>. Protein alteration (vulcanization of proteins). |
| **9.** | MMP-9. ROS | Islet<br>Endothelial cell disconnect?<br>Beta cell disconnect?<br>Apoptosis? |
| **10.** | ROS (reactive oxygen species) - "(Toxic oxygen)" ROS beget ROS | "Rusting of the islets"<br>[Cell membrane damage]<br>{Apoptosis} |

T1DM 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 $H_2O_2$ through two pathways: the first is the 1,2-enolization pathway which leads to 3-deoxyglucosone forming $H_2O_2$ 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 $H_2O_2$ and carboxymethyllysine.

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. AGEs also play a direct role in the development of islet amyloid as it promotes its formation (see section on amylin and amylin derived islet amyloid). 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 [20, 21, 22, 23].

The multiligand immunoglobulin superfamily 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 possibly to accelerated prediabetic and diabetic atherosclerosis (atheroscleropathy) [24]. RAGE has also been shown to act as a receptor for amyloid and, in turn, amyloid acts to induce signal transduction which indicates that RAGE may be a potential target

for inhibiting the accumulation of amyloid and its associated cellular dysfunction (inflammation and redox stress) [25].

## (A). Antioxidant Reserve (Defense Mechanisms) – Compromised.

In addition to the excess generation of the ROS seen in diabetes, there exists an impaired generation of endogenous antioxidants. Superoxide dismutase (SOD) [26], glutathione reduced (GSH) [27], and ascorbic acid (Vitamin C) [28] are all decreased and associated with atheroscleropathy 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) [29]. The exact mechanisms 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 [30].
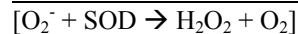
## (A). Absence of Network Antioxidant Enzymes

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 eNOS. This implicates eNOS not only in the endothelial

**Table 6.** Antioxidants: catalytic/enzymatic inactivation of free radicals.

**Enzymatic antioxidants**
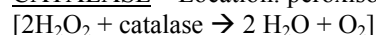
SUPER OXIDE DISMUTASE (SOD) – Location: mitochondrion
$[O_2^- + SOD \rightarrow H_2O_2 + O_2]$
ecSOD (extracellular)
MnSOD (mitochondrial)
CuZnSOD (intracellular)

CATALASE – Location: peroxisome
$[2H_2O_2 + catalase \rightarrow 2 H_2O + O_2]$

GLUTATHIONE PEROXIDASE – Location: mitochondrion/cytosol
(Glutamyl-cysteinyl-glycine tripeptide) glutathione reduced –SH to the oxidized disulfide GSSG.
(Glutathione peroxidase) $[GSH + 2H_2O_2 \rightarrow GSSG + H_2O + O_2]$
(Glutathione reductase) $[GSSG \rightarrow GSH]$ at the expense of $[NADH \rightarrow NAD^+]$ and/or $[NAD(P)H \rightarrow NAD(P)^+]$
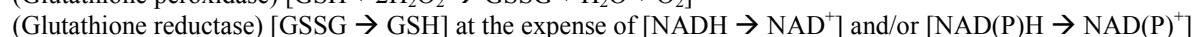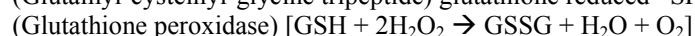
NOS (nitric oxide synthase). – Location: membrane
Isoforms:
(e)NOS (endothelial): good
(n)NOS (neuronal): good
(i)NOS (inducible-inflammatory): bad

$O_2^-$ and nitric oxide (NO) are consumed in this process with the creation of reactive nitrogen species (RNS).
$O_2^- + NO \rightarrow ONOO^-$ (peroxynitrite) + tyrosine $\rightarrow$ nitrotyrosine.
Nitrotyrosine reflects redox stress and leaves a measurable footprint.
NO: the good; $O_2^-$: the bad; $ONOO^-$: the ugly [81]
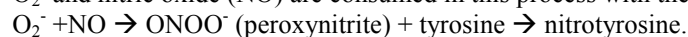
**Nonenzymatic antioxidants**

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.

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 [31]. This represents the loss of the naturally occurring free oxygen radical scavenging antioxidant effect of endothelial nitric oxide (eNO) (Table 6).

If any one of the antioxidant enzymes 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. There is evidence of a gene polymorphism in humans and recently Miyamoto *et al.* [32] 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, hypertension or even amylin derived islet amyloid (ADIA) deposition. As the human genome evolves, we are certain to find other alterations in various populations throughout the world.

## (A). Ageing

Ageing has been shown to be associated with an increased risk of developing T2DM and correlates to the development of islet amyloid. 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 eNO associated with aged cells creates a deficiency of the naturally occurring antioxidant eNO [33]. 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 [34, 35].

## (F). Free Fatty Acids

Free fatty acid (FFA) elevation is known to be associated with insulin resistance, metabolic syndrome 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 Aassociation 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 [36].

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 [36, 37]. 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.* [37] 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 [37].

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 overutilized 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 insulin resistance, metabolic syndrome and T2DM. There is an associated defect of the 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 accelerated atherosclerosis and coronary heart disease as well as with increased redox stress [38, 39, 40].

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 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 [41, 42].

## (L). Lipotoxicity - Specific

Lipotoxicity is also associated with insulin resistance, metabolic syndrome and T2DM. Unger *et al.* [43, 44, 45, 46, 47, 48] feel this specific lipotoxicity is attributed to products of the excessive non-beta- (non-mitochondrial) 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 insulin resistance, obesity, cardiovascular disease and T2DM by creating cellular dysfunction and, in time, promoting programmed cellular death (lipoapoptosis) [45, 46].

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, resulting in lipoapoptosis,

include triglycerides, ceramide, and products of lipid peroxidation. Ceramide (an amino alcohol with a LCACoA 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 [49]. Ceramide can be formed in these cells by direct *de novo* synthesis from FFAs [45]. Ceramide increases inducible nitric oxide synthase (iNOS) through the activation of NFkappa B [50] resulting in increased NO [51] which forms the potent damaging oxidants, ROS and RNS (such as peroxynitrite responsible for the lipoapoptosis of these cells) [52, 53]. This apoptotic pool of beta cells eventually overwhelms the primordial periductal replicative pool resulting in not only beta cell dysfunction but also beta cell loss with an associated decreased availability of insulin.

In autoimmune T1DM, the cytokine-mediated apoptosis of beta cells is indiscriminate and usually complete as a result of the ceramide, NFkappa B, iNOS, increased NO and ONOO$^-$ mechanism resulting in insulin dependence. Whereas, 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 which incorporates and implicates the multiplicative manifold A-FLIGHT toxicities).

## (I). Insulin Toxicity

Insulin toxicity (hyperinsulinemia, hyperproinsulinemia) is associated with insulin resistance, metabolic syndrome and early T2DM. Insulin is known to up-regulate the number of AT-1 receptors, activate the RAAS, and be capable of crosstalking 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 within the islet as insulin, proinsulin and amylin are all three elevated within the islet milieu [10, 11, 12, 13, 14, 15].

## (I). Inflammation Toxicity

Inflammation toxicity may be associated with increased redox stress and cytokines associated with insulin resistance, metabolic syndrome, and early, as well as late, T2DM. The inflammatory mediators, TNFalpha and interleukin 6, are closely associated with central obesity in insulin resistance, metabolic syndrome and T2DM [54, 55, 56].

Elevated white blood cell count, fibrinogen, sialic acid, orosomucoids and highly sensitive C reactive protein are associated with the development of T2DM.

Factor VIII, von Willebrand factor and activated partial thromboplastin time have also been implicated in the development of T2DM [57]. NFkappa B is associated with redox stress and iNOS in the apoptosis of the beta cell in both T1DM and T2DM. Both NFkappa B and TNFalpha are induced by ROS [58]. 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 $H_2O_2$ and the $OH^-$ radical but not super oxide is involved. ROS treatment of endothelial cells induces the focal adhesion kinase pp 125 PAK, a cytosolic tyrosine kinase which has been implicated in the oxidant-mediated adhesion process [58]. This section and the section "(A). Ageing" are closely related as ROS and RNS are widely implicated in the inflammatory and ageing process [59].

## (G). Glucotoxicity

Glucotoxicity is associated with both type 1 and type 2 diabetes mellitus and, thus, the similarly shared multiple opathies associated strongly with redox stress (Figure 2).

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. The reduced oxygen products formed are $O_2^-$, $OH^-$, and $H_2O_2$. Each of these ROS can contribute to damaging lipids and proteins through cross-linking and fragmentation [60, 61, 62]. The process of combined autoxidation and glycation are 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].

Autoxidation 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.

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 and maintain an 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 $NAD^+$/$NAD(P)^+$. Sorbitol is then converted to fructose by sorbitol dehydrogenase at the expense of $NAD^+$ $NAD(P)^+$ being converted to NADH/NAD(P)H [63, 64, 65].

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 be titled "Islet 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 [66]. 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 and fibrosis (remodeling) within pancreatic islets [67].

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 [68] 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 insulin resistance, metabolic syndrome, and T2DM, and both contribute to the excess redox stress associated with the other A-FLIGHT toxicities, having an overall multiplicative effect within the islet on the beta cell [68].

## (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 ($O_2^-$) generating enzyme [16].

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) [69]. 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, in the near future, amylin levels may become a screening tool for the development of essential hypertension [70]. 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 [71, 72].

## (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 [73, 74, 75]. This gene polymorphism is especially important in those individuals with a decrease in dietary folate. Hyperhomocysteinemia can be improved with folate supplementation and can improve endothelial-dependent endothelial cell dysfunction [76].

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 [77]. This plays an extremely important role for those diabetic patients on dialysis [78].

Hyperhomocysteinemia 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 thiol and reducing glutathione pools and increasing GPx activity with restoration of the endothelial function [79].

The ensuing cellular redox stress is magnified and total homocysteine consumes NO by the indirect process of $O_2^-$ converting NO to toxic peroxynitrite ($ONOO^-$). In addition to $ONOO^-$ formation, NO in conjunction with thiols and oxygen radicals generates nitrotyrosine and nitroarginine which compete for the substrate eNOS in a feedback mechanism, limiting further NO generation [80, 81, 82]. As a result, there is endothelial cell dysfunction, endothelial cell toxicity and endothelial cell loss, increased ROS, increased $ONOO^-$ and decreased NO associated with hyperhomocysteinemia [83].

Hyperhomocysteinemia is multiplicative in nature and even though the effects of hyperhomocysteinemia may occur later in T2DM than in 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 hyperhomocysteinemia.

A recent clinical study by Maejima *et al.* [84] 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.* [85] 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 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 [86].

### (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 [87]. 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 [88].

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.

### AMYLIN AND AMYLIN DERIVED ISLET AMYLOID (See previous "(A)." sections)

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 a fraternal twin of insulin.

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 is the monomeric substrate unit of the polymer ADIA which forms between beta cells, and between beta cells and endothelial cells within the islet. ADIA creates a space-occupying lesion which functions as a diffusion barrier within the islet and is responsible for both a secretory and an absorptive defect to secretion of insulin and absorption. ADIA is present on histological examination of pancreatic islets by Congo red staining in at least 70% (or more) of T2DM patients [2]. The presence of amyloidosis within the islets correlates to the duration and the severity of T2DM. Hyperinsulinemia and, therefore, hyperamylinemia is associated with insulin resistance, metabolic syndrome and T2DM (Table 8).

The damaging role of redox stress within the islet may contribute to the unfolding of the native secondary structure of beta cell-derived amylin. Once unfolded, this molecule has the amyloidogenic potential to refold into the anti-parallel crossed beta-pleated sheet structure of ADIA (Figure 4).

ADIA develops diffusely throughout the pancreas before becoming severe, and totally disabling the endocrine pancreas [89]. A recently published article by Sakuraba *et al.* [90] was able to demonstrate that, in T2DM patients, there is increased oxidative stress-related tissue damage correlated with the extent of islet amyloid beta cell lesions. They were able to demonstrate a reduced expression of SOD associated with islet amyloid and a decrease in beta cell mass. These findings show that oxidative stress (with associated reduced expression of SOD) is related to islet amyloid, decreased beta cell mass and beta cell volume density.

Amylin stimulates lipolysis in vivo and may be a possible mediator of induced insulin resistance. Ye *et al.* [91] 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

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**Table 7.** The RAAS acronym.

**For the prevention, treatment and stabilization of:**

I. The vulnerable pancreatic islet in T2DM
II. Arterial vessel wall remodeling in atherosclerosis
III. The glomerulus in T2DM diabetic nephropathy
IV. The dysfunctional endothelial cell

**R** **R**eductase inhibitors (HMG-CoA). Decreasing modified LDL cholesterol, i.e. oxidized, acetylated LDL cholesterol. Improving endothelial cell (EC) dysfunction. Thus, decreasing the oxidative stress to the arterial vessel wall and the islet. Redox stress reduction.

**A** **A**CEi-prils. **A**RBS-sartans. Both inhibit the effect of Angiotensin II locally as well as systemically. Affecting the 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 anti-inflammatory effect within the islet. **A**SA antiplatelet anti-inflammatory. Redox stress reduction.

**A** Aggressive control of diabetes and homocysteine. Decreasing modified LDL cholesterol, i.e. glycated LDL, glycoxidated and homocysteinated LDL cholesterol. Improving endothelial cell dysfunction. Also decreasing glucose-homocysteine toxicity and to the intima and pancreatic islet. Redox stress reduction.

**S** **S**tatins. 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 anti-oxidant effect within the islet promoting stabilization of the unstable, vulnerable islet. Redox stress reduction.

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 [91]. 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 beta cell dysfunction within the islet.

There are amylin binding sites within the renal cortex and amylin activates the RAAS with elevations in renin and aldosterone [14].

Islet amyloid deposition within the islet is accelerated by advanced glycosylation of IAPP [92]. These findings suggest that glucotoxicity resulting in AGE formation both promotes and accelerates deposition of ADIA as well as increasing oxidative stress [93].

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 [94]. This finding is of extreme importance in understanding the damaging role of ADIA and is additive to the A-FLIGHT toxicities of insulin resistance, metabolic syndrome and T2DM.

To date, there have been no longitudinal studies in humans such as the epic serial histopathologic studies of the non human primate *Macaca nigra* done by Howard [95, 96]. He was able to show that continued amyloid deposition was associated with a further reduction in insulin secretion and deterioration in intravenous glucose tolerance. Moreover, he demonstrated that fasting hyperglycemia was a late phenomenon and occurred in these animals only with the significant deposition of islet amyloid which we now know is ADIA (it would be interesting to longitudinally follow the trend of humans having impaired glucose tolerance evolving into impaired fasting glucose and, subsequently, overt T2DM with ^{123}I labeled serum amyloid P scintigraphy in order to follow the development of ADIA with the
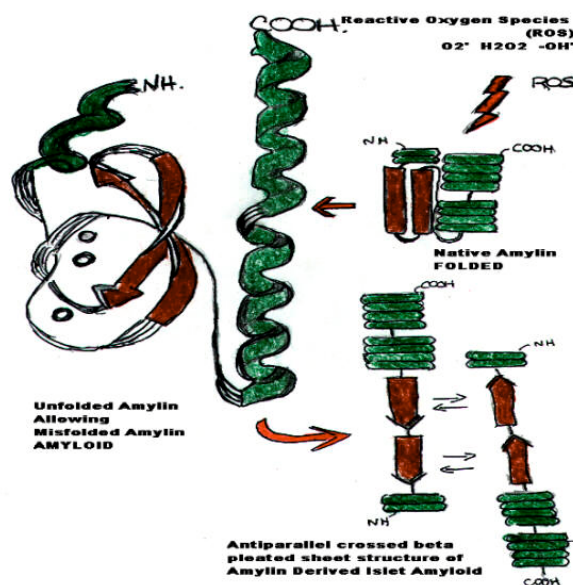


**Figure 4.** Misfolded proteins within the islet: islet amyloid. The damaging effects of ROS (reactive oxygen species) may cause unfolding of the tightly folded native amylin secondary structure. Once unfolded, the amyloidogenic amylin will form the anti-parallel crossed beta pleated sheet structure of the islet amyloid. The alpha subunits are colored green and the beta subunits are colored rust forming the secondary structure.

associated metabolic perturbations of insulin resistance, metabolic syndrome and T2DM).

A nonsense mutation in the IAPP (amylin) gene resulting in a glycine for serine substitution at position 20 of the primary structure of IAPP (amylin), S20G mutant amylin was found to be associated with early-onset T2DM in a Japanese population in 1996 [97]. A larger study performed in 2001 was found to be supportive and the S20G mutation was found in 40 (2.6%) of 1,538 patients with unrelated T2DM and 9 (0.8%) of 1,108 non diabetic controls (P=0.0007) [98]. In 2000, Sakagashira *et al.* were able to demonstrate increased amyloidogenicity and intracellular cytotoxicity of S20G [99] and, in 2001, Ma *et al.* were able to demonstrate that full-length and truncated IAPP S20G formed more amyloid-like fibrils and did this faster when compared to wild type IAPP [100]. There may well be other important nonsense mutations in other specific populations as well and only time will tell as the human genome progresses and specific screenings are performed.

**Table 8.** The natural history of type 2 diabetes mellitus.

I. **LATENT STAGE:** *[EARLY]*

**Insulin Resistance:**
- Genetic Component
- Environmental component: obesity (modifiable) - Sedentary life style: ageing (nonmodifiable)

**Beta Cell Defect:** (Dysfunction)
- Genetic ....... Abnormal processing, storage or secretion
- 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]**
- Increased insulin resistance [Feeds forward] > Glucotoxicity [Feeds forward] > Insulin resistance [Feeds forward] > Glucotoxicity: creating a vicious cycle
- Islet amyloid. Increasing beta cell defect. Loss of beta cell mass with displacement. [Remodeling of islet architecture including ECM]. Beta cell loss centrally

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]*
- Change in treatment modality. Start treatment at stage III-IV (IGT-IFG) Va. Vb. Vc. Mild/Moderate/Severe/Complete. Use medications that do not increase insulin or amylin. Use combination therapy
- Paradigm Shift. Start treatment at the earlier stage of IGT

# REDOX STRESS AND MATRIX METALLOPROTEINASES

Elevated redox stress is associated with an increase in matrix metalloproteinase (MMP) activity, especially the inducible MMP-9 [101].

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 [101].

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 peroxynitrate 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.

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 ADIA fibrils to the more intermediate size toxic amyloid particles and contribute to apoptosis as described by Janson *et al.* [94] (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 dermal lamina [102].

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 islet, and how these two interact with the multiplicative effect of the A-FLIGHT toxicities of insulin resistance, metabolic syndrome, amylin, hyperamylinemia, ADIA and T2DM. 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 atherosclerosis, chronic inflammatory diseases (pancreatitis, rheumatoid arthritis, ulcerative colitis, Crohn's), ageing, cancer, arterial vessel wall in atherosclerosis, ischemia/ischemia-reperfusion injury, hypertension, diastolic/systolic dysfunction, congestive heart failure, nephropathy and neurodegenerative diseases.

When redox cycling (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 [58, 103].

## REFLECTIONS AND FUTURE DIRECTIONS

An article entitled "Diabetes as a manifold disease" which had previously been 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 [104, 105]. T2DM remains a manifold disease not only in its etiology but also in its manifold toxicities associated with insulin resistance, metabolic syndrome and T2DM. In 1902, the author (unknown) could not have envisioned the exponential growth of T2DM 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 islet redox stress and the rusting of the beta cell within vulnerable pancreatic islets.

It is readily apparent that the treatment and potential prevention of this disease can be accomplished through global risk reduction of the manifold toxicities by using currently

available treatment modalities of aggressive dietary and exercise programs which were shown, in the Diabetes Prevention Program (DPP) [106] and the therapeutic plan put forth with the RAAS acronym for the treatment and prevention of T2DM (Table 7) to significantly reduce (by 58%) the relative risk of developing T2DM.

Newer treatment modalities being developed which include the inhaled form of insulin Exubera[TM] [107] and the buccally absorbed Oralin[TM] [108] (*vs.* injectable insulin) will undoubtedly aid the clinician and the patient in this daunting task of saving the vulnerable islet while preventing the rusting of the beta cell by means of earlier diagnosis and global risk reduction in T2DM.

## AUTHORS' ADDITIONAL NOTE

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The authors point out that intermediate sized amyloid particles induce apoptosis by membrane disruption and referenced the findings of Janson *et al.* [94].

The authors would like to point out to the readers of this article that there is evidence that all amyloid (including amylin derived islet amyloid) are toxic to cells via a common oxidative mechanism.

Schubert *et al.* [110] were able to demonstrate nicely that amyloid is cytotoxic to clonal and primary cells via a free radical pathway with accumulation of $H_2O_2$ and this toxicity was inhibited by the NAD(P)H oxidase inhibitors diphenylene iodonium (DPI) and vitamin E and spin trap agent N-tert-butyl-alpha-phenylnitrone-trichroromethyl (BPN).

Thus, amylin derived islet amyloid may be induced and accelerated by oxidative stress and once formed may be responsible for beta cell death through the process of oxidative stress.

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