

“A” is for Amylin and Amyloid in Type 2 Diabetes Mellitus

Melvin R Hayden^{1,2}, Suresh C Tyagi³

¹Department of Cardiovascular Atherosclerosis, Metabolism and Aging, Camden Community Health Center, Camden, MO, USA. ²Preceptor in Family Medicine, Department of Family and Community Medicine, University of Missouri Columbia, MO, USA. ³Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS, USA

Summary

Amyloid deposits within the islet of the pancreas have been known for a century. In 1987, the islet amyloid precursor polypeptide (IAPP) amylin (a 37 amino acid) was discovered. Recently there has been an explosion of amylin's importance in the development of type 2 diabetes mellitus (T2DM). This review is intended to share what is understood about amylin derived amyloid and the role it plays in T2DM. Whether islet amyloid is an epiphenomenon, a tombstone, or a trigger it leaves an indelible footprint in greater than 70% of the patients with T2DM. There is current data supporting the damaging role of intermediate sized toxic amyloid particles to the beta cell resulting in a beta cell defect which contributes to a relative deficiency or loss of insulin secretion. Within the islet there is an intense redox stress which may be associated with the unfolding of amylin's native secondary structure compounding its amyloidogenic properties. In addition to the beta cell defect there may be an absorptive defect as a result of amyloid deposition in the basement membranes which form an envelope around the intra-islet capillary endothelium. We have an opportunity to change our current treatment modalities with newer medications and we should attempt to diagnose T2DM

earlier and use these newer treatment strategies in combination to decrease glucotoxicity without elevating endogenous insulin and amylin. In the 21st century our goal should be to prevent remodeling, save the pancreatic islet, conquer islet amyloid, and amyloid diabetes.

Historical Background and Introduction

2001 marks a century since Eugene L Opie first described the presence of a hyaline staining substance currently referred to as islet amyloid and noted its association with diabetes mellitus (Figure 1) [1]. In 1869, Paul Langerhans was the first to describe the endocrine pancreas and how these bundled cells appeared to be

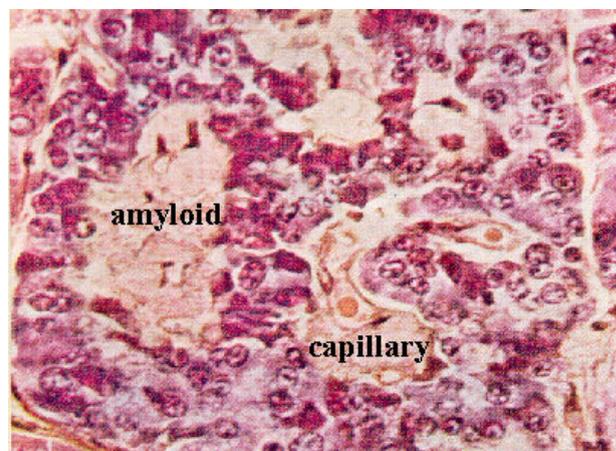


Figure 1. Islet amyloid.

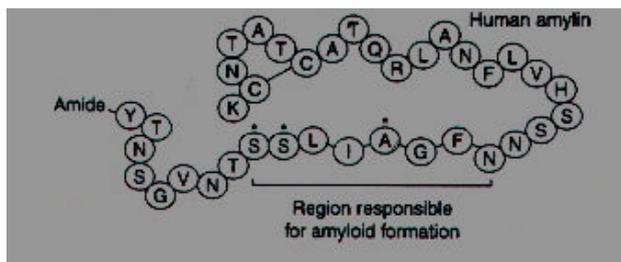


Figure 2. The primary structure of amylin. (with permission of Dr. B Ludvik).

suspended and unconnected in an ocean of acinar cells (exocrine pancreas). Laguesse in 1893 named these mysterious cells the islands or islets of Langerhans (îles de Langerhans) to honor his colleague. Oskar Minkowski in 1889 made the discovery that connected the pancreas and diabetes in his depancreatized dogs [2]. In 1901, while at Johns Hopkins University, Eugene L Opie supplied a missing link by showing a pathological connection between diabetes and hyaline degeneration within the islet Langerhans had previously described.

The amyloid nature of this hyaline material was established by Ahronheim in 1943 and confirmed by alkaline Congo red staining by Ehrlick JC and Ratner IM in 1961 [3, 4]. Westermark P in 1973 was able to demonstrate the fibrillar structure with the use of electron microscopy [5].

By 1987, two contemporary investigators (Westermark P and Cooper GJS) in separate laboratories discovered that this hyaline staining material consisted of a 37 amino acid monomer referred to as islet amyloid polypeptide (IAPP) by Westermark P and named amylin in 1988 by Cooper GJS (Figure 2) [6, 7].

Currently, there is a need to become more familiar with the relation of amylin's overproduction and abnormal processing, storage and/or secretion with subsequent amylin derived amyloid deposition resulting in an alteration in structure and function within the islet and the associated beta cell dysfunction seen in type 2 diabetes mellitus (T2DM).

On January 26th, 2001 the Center for Disease Control in Atlanta, Georgia released to the major television networks world news that we are in the midst of an epidemic of pandemic proportions regarding T2DM. Due to the exponential growth of T2DM globally we will have to acknowledge, as did Opie, the disease process of remodeling of the endocrine pancreas associated with T2DM [8].

Amyloid

We were instructed in medical school to include amyloidosis in our differential diagnosis for those patients with unexplained organ failure such as cardiac, hepatic, and renal failure. We were also told that we would not be expected to see many cases of amyloidosis unless we were cardiologists, nephrologists, or hematologists. Currently, this dictum should be pondered as T2DM and Alzheimer's disease (AD) are two very common diseases that are growing exponentially as our society ages and they share a commonality. Amyloid is the common thread interweaving the two and is central to their origins and transforming histological changes. There may even be an association between them as Gregg EW and Narayan Venkat KM have suggested in their article [9]. We may need to add to our list of diabetic -opathies the term "cognopathy".

Islet amyloid is considered by many to be an epi-phenomenon. Could amyloid be a "tombstone" or a "trigger" or a combination of both in regards to these two exponentially growing diseases? [10]. Whether or not it is causal (a trigger), or a bystander (a tombstone) or a combination we know that islet amyloid is being deposited in up to 70-90% of patients with T2DM and we must continue to study this phenomenon and better understand its plight [11, 12]. Amyloid is literally defined as being "starchlike" from the Greek root word *amylō* because these areas turned blue when iodine was applied to the tissue. This definition however is a misnomer as amyloid is a

proteinaceous extracellular deposit resulting from the polymerization of polypeptides which undergo aggregation into antiparallel crossed beta pleated sheets. A characteristic feature of amyloid histologically is the positive staining with Congo red and birefringence on viewing with polarized light. Electron microscopy reveals interlacing bundles of parallel arrays of fibrils with a diameter of 7-10 nanometers (Figure 3). X-Ray diffraction reveals the adjacent amyloid fibrils to be organized as antiparallel crossed beta-pleated sheets. Amyloid is classically made up of the following.

Fibrillary (polypeptide) protein: Amyloidogenic polypeptides. Each form of amyloid has its own unique fibrillary polypeptide structure and these individual polypeptides serve as a monomeric unit of the polymerized aggregated beta-pleated sheet structures. In this article we are discussing the unique polypeptide amylin a 37 amino acid structure. Alzheimer's disease as another example contains the A beta 40 and 42 amino acid polypeptide monomer (Figure 3).

Amyloid P component. Stacks of pentagonal donut-shaped proteins consisting of serum amyloid P (SAP) which are related to the acute phase reactants: Serum amyloid A (SAA) and C-reactive protein (CRP) both being synthesized in the liver. This component contributes to the stability of the amyloid fibrils (Figure 3).

Glycosaminoglycans (GAGs). Specifically the heparan sulfate proteoglycan (Perlecan). This molecule is thought to be responsible for the iodine staining properties of amyloid and is responsible for amyloid binding to the basement membranes. There is no structural element as it is part of the matrix of amyloid (Figure 3).

Apolipoprotein E. Contributes to the stability of the amyloid fibrils and thus the importance of

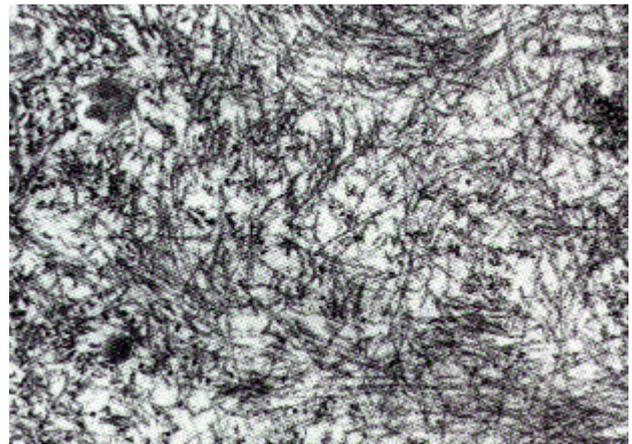


Figure 3. Electron micrograph of amyloid.

the Apo E allele polymorphism (epsilon 4) associated with Alzheimer's disease. This component also contributes to the stability of the amyloid fibrils. There is no structural element as it is part of the matrix of amyloid (Figure 3).

Amyloidosis is characterized by proteinaceous tissue deposits with common morphologic, structural, and staining properties but with variable protein (polypeptide) composition. The older clinical classification of amyloid includes: Primary, Secondary, Familial, and Isolated forms of amyloid formation.

The newer classification is dependent on the polypeptide or protein composition of the amyloid (Table 1).

Amylin or Islet Amyloid Polypeptide (IAPP)

Cooper GJS in 1988 was responsible for giving the name "amylin" to islet amyloid polypeptide [13]. Amylin and islet amyloid polypeptide are currently interchangeable terms for the 37 amino acid polypeptide which forms the monomeric unit of polymerized, aggregated, and beta pleated sheet structure of islet amyloid (Figures 1, 2, 3).

Amylin is co-synthesized, co-packaged within the Golgi apparatus, and co-secreted within the secretory granule by the islet beta cell in response to elevations of plasma glucose.

Amylin may be referred to as insulin's "fraternal twin" as it is constitutively expressed with insulin when exposed to non-glucose and glucose stimulation (nutrient stimuli). The amylin gene is located on the short arm of chromosome 12 and transcribes an 89 amino acid precursor peptide (Figure 4) [14]. Proprotein convertases (PC1, PC2, and PC3) are responsible for the processing of the prohormones to the active secreted hormones insulin and amylin. It is primarily PC2 that is responsible for amylin processing within the secretory granule and is responsible for converting the prohormone (89 amino acid) to the actively secreted (37 amino acid) amylin [15]. Since amylin's discovery in 1987 it is

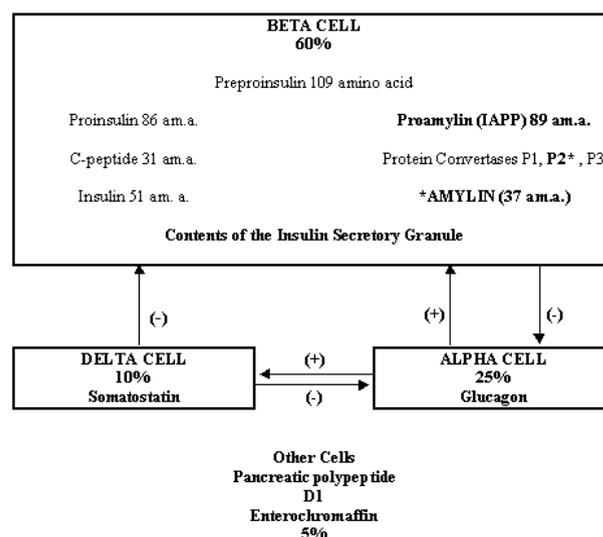


Figure 4. Interrelationships of the islet cells.

Table 1. Classification of amyloid.

Amyloid designation	Protein (polypeptide)	Associated diseases	Clinical classification
AA	SAA serum amyloid A	[Recurrent inflammation] R.A., U.C. , Crohn's disease Hodgkins Cancer Med. Fever Many others	Secondary Secondary Secondary Secondary Familial
A beta Beta Amyloid	Beta Amyloid Polypeptide	Alzheimer Disease Downs Syndrome	Isolated Isolated
AE E Endocrine	Procalcitonin	Medullary Ca Thyroid	Isolated
AIAPP	Amylin Islet Amyloid Polypeptide	Type 2 Diabetes Mellitus Pancreatic Islet Cell Tumor	Isolated ? Isolated
AF	Serum Prealbumin	Familial A. Polyneuropathy	Familial
AL	kappa / lambda	Multiple Myeloma 20%	Primary, Primary idiopathic*
ASc		Cardiac Amyloid Systemic senile amyloidosis	Isolated Senile
ATTR	Transthyretin	Senile systemic cardiac	
HA (A beta2M)	beta 2 Microglobulin	Long standing dialysis	Secondary
IAA (AANF)	Atrial Naturetic Peptide	Isolated Atrial Amyloid	Isolated
HCCAA Acys AP	Cystatin-C Serum Amyloid P	Familial Icelandic Amyloid Angiopathy. Present in all amyloid	Isolated
Prion Amyloid (AprPSc)	PrPSc	Scrapie / KURU Creutzfeldt - Jakob Disease Gerstmann - Straussler Syndrome vCJD transmissible Bovine SE	Isolated Isolated Isolated Isolated

currently thought to be the third active pancreatic islet hormone important in glucose homeostasis. It potently inhibits gastric emptying and is important in controlling and delaying the rate of meal derived glucose. It inhibits hepatic release and production of glucose in the postprandial period. In addition to the above amylin has been shown to inhibit glucagon secretion as well as somatostatin. Amylin's synthesis and excretion parallels insulin in the beta cell.

Amylin levels are elevated in the type 2 diabetic patient, the insulin resistant obese patient, and the patient with impaired glucose tolerance (Figure 4) [16]. In addition to producing satiety, amylin also increases thirst which indicates it has an action within the central nervous system [17, 18, 19, 20]. Amylin has been shown to have binding sites within the renal cortex in the area of the juxtaglomerular apparatus. Amylin has been shown to activate the rennin angiotensin aldosterone system [21, 22]. In 2001, pramlintide, a human amylin analog, may come to market to use in type 1 diabetes mellitus and insulin requiring T2DM patients. Since the time of Starling many have chosen to treat hormonal deficiencies with replacement of human hormones. Thyroid extract being the first and insulin by injection being the second to replace the deficient hormone state. Beta cell derived pancreatic hormonal replacement has not been undertaken since 1921-1922 when Banting and Best discovered the "internal secretion" insulin.

In 1988 following the discovery of amylin and sequencing the peptide Garth Cooper named this second beta cell derived hormone amylin. Amylin is now thought to be the third active pancreatic hormone important in glucose homeostasis. Cooper was a co-founder of the San Diego, California based company named Amylin Pharmaceuticals in 1987.

They developed a human amylin analog as human amylin formed amyloid in vitro and turned out to be unsuitable for human

administration as a replacement for this missing hormone [personal communication with Andrew Young VP, Research Amylin Pharmaceuticals Inc., San Diego, CA, USA]. Amlintide [23] was renamed (for its proline substitutions at positions number 25, 28, and 29) "Pramlintide" which prevented the in vitro amyloid formation. Currently, pramlintide is being reviewed by the FDA and this human amylin analog may come to market during this year 2001, a century after its human counterpart was first described by Opie. Multiple phase III studies have shown it to be effective in lowering hemoglobin A glycosylated-c (HbA1c) values and preventing weight gain as compared with insulin replacement (there are no cited journal articles to be found to date). In addition to replacing this missing beta cell derived islet hormone in insulin requiring diabetics, we as clinicians, will need to become more familiar with the relation of amylin's overproduction and abnormal processing, storage and/or secretion with subsequent amylin derived amyloid deposition resulting in an alteration in structure and function within the islet and the associated beta cell dysfunction seen in T2DM.

Regarding the explosion of interest in amylin, there are over 900 entries on a PubMed search using the single search word amylin. In 1998-1999 there were just over 100 entries resulting in a nine fold increase in approximately 3 years. As research interests continue we will undoubtedly elucidate more mechanisms of action of this fascinating polypeptide.

In recent phase III clinical trials the human amylin analog (pramlintide) was shown to reduce HbA1c values and result in a decrease in weight as compared to insulin treated control subjects which increased weight gain. The rodent model and human model have been important in adducing the role of amylin. The rat model [24, 25] and the human islet amyloid polypeptide (hIAPP) transgenic mouse model [26, 27, 28, 29, 30] have proved to be

invaluable regarding our current knowledge and will undoubtedly serve to elucidate future actions of the polypeptide amylin.

Amylin's Role Associated with Other Type 2 Diabetogenic Factors

Each of us are given approximately 1 to 1.5 million islets and the figure remains stable when there is proper homeostasis between the replicative pool, the senescent pool and apoptosis. With insulin resistance, impaired glucose tolerance, and T2DM there is loss of this homeostasis with excessive loss by apoptosis due to the amyloidogenic toxicity associated with the unfolded polypeptide amylin [31]. We can lose approximately one half of these islets and still maintain homeostasis. When we reduce the number of properly functioning beta cells glucose homeostasis may become impaired. As the beta cell mass continues to further decline in number and function we see the progressive development of impaired glucose tolerance and with even further decline the development of overt T2DM. Recently, there have been two reports published in the February 2001 Diabetes Supplement entitled "Birth, Life, and Death of a Beta Cell in Type 2 Diabetes" that demonstrated no significant loss in beta cell mass in T2DM patients. These two separate authors thus emphasize that beta cell dysfunction may be more important than beta cell loss. They were able to demonstrate that the beta cell was capable of being replaced through the process of replication from the pancreatic ductal cells within the acinar portion of the pancreas [32, 33]. The loss of homeostasis of the various pools (replicative, senescent, and apoptotic) and the functional and dysfunctional pools of beta cells remain to be more fully elucidated.

As the beta cell mass and function continue to decline we see the progressive nature of T2DM in and of itself that was exemplified by the findings in the United Kingdom Prospective

Diabetes Study (UKPDS) of progressive elevations of HbA1c regardless of the treatment modality [34]. This decrease in beta cell islet composition was nicely described in the epic study of serial histopathologic findings during the progression of diabetes in the *Macaca Nigra* monkey by CE Howard Jr in 1986. He was able to demonstrate that the loss of the beta cells were primarily within the center of the islet with the peripheral alpha and delta cells remaining preserved indicating that there may be a microvascular component to the beta cell loss [35].

The afferent arteriole of the islet preferentially enters centrally and percolates to the efferent collecting venules located on the outer mantle while the numerous capillaries within the islet simulate the renal glomerulus (Figure 5) [36, 37].

T2DM is a multifactorial, polygenic disease associated with at least three diabetogenic factors interacting to result in the development of T2DM: genetic, environmental, and endogenous (islet amyloid) factors (Figure 6). The beta cell defect and insulin resistance are both genetically predetermined and interact with environmental and ethnic cultural conditions as well as the endogenous diabetogenic factor: islet amyloidogenic amylin (Table 2).

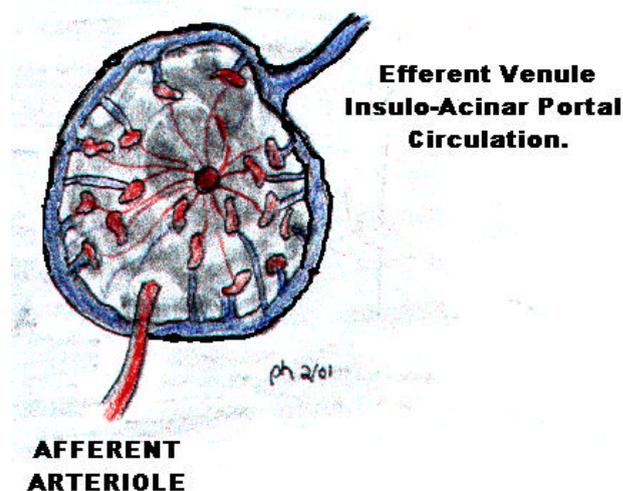


Figure 5. Islet microcirculation. "Core to mantle".

Table 2. The five stages of T2DM: historical time line of T2DM.

I. (LATENT) TYPE 2 DIABETES MELLITUS PREDIABETES: [EARLY]

Insulin Resistance: (Figure 5)

- Genetic Component.
- Environmental Component. Modifiable: Obesity / Sedentary life style; Nonmodifiable: Ageing

Beta Cell Defect: (Dysfunction)

- Genetic..... Abnormal processing, storage, or secretion.
- Intracellular extracellular amylin fibril toxicity [23]. 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 islet amyloid forms an envelope around the capillary. This is in addition to the increase in basement membrane associated with the pseudohypoxia (associated with glucotoxicity) and the redox stress within the capillary (Figure 7)

II. PROGRESSIVE: [MIDDLE]

Persistent Hyperinsulinemia

Persistent Hyperamylinemia

- Continued remodeling of endocrine pancreas (**amyloid**).
- Beta cell displacement, dysfunction, mass reduction, and diffusion barrier.

III. IGT (Impaired Glucose Tolerance): [LATE]

[Start treatment at this time]

[Diagnose earlier: Rejuvenation of the 2 hour glucose tolerance blood sugar 140-199 mg/dL]

- 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 [35]

IV. IFG (Impaired Fasting Glucose): [LATER]

[Blood sugar ranging 110-126 mg/dL]

[Impaired hepatic glucose production (HGP)]

- Increasing global insulin resistance (HEPATIC) with subsequent gluconeogenesis. Feeding forward in the vicious to accelerate insulin resistance globally.

V. (OVERT) TYPE 2 DIABETES MELLITUS: [TO LATE]

- Change in treatment modality: Start treatment at stage III-IV (IGT) (Figure 5).
 - Paradigm Shift. Va. Vb. Vc. Moderate. Moderate/Severe. Severe
[Start treatment at the earlier stage of IGT. Use medications that do not increase insulin or amylin. Use combination therapy. Treat aggressively to known goals]
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Future Directions and Changes in the Treatment Paradigm for Type 2 Diabetes Mellitus

When amylin was discovered in 1987 and its pathologic relation to T2DM revisited from

1901, there were basically no changes to make in the treatment of T2DM as we only had sulfonylureas and exogenous insulin which patients were reluctant to self administer preferring the oral route of treatment. Since 1995 we have had the introduction of many

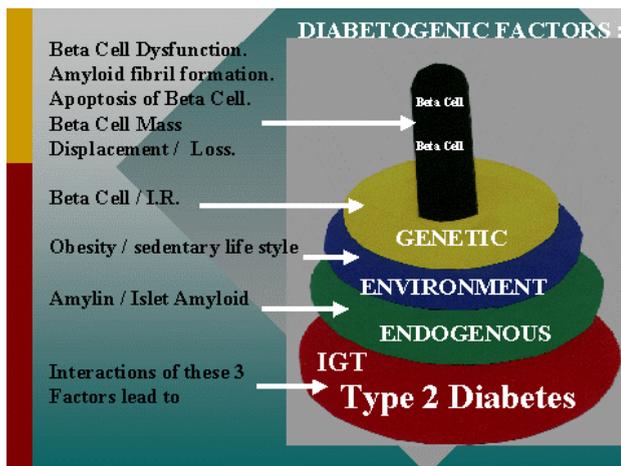


Figure 6. Child's stacking toy model.

new oral treatment modalities. In addition, we have been able to better delineate the multiple toxicities associated with T2DM. The following mnemonic can be used as an aid to remember these toxicities. Each of these toxicities are associated with the production of reactive oxygen species (ROS) which may be important to the unfolding of the native secondary conformational structure of the amylin polypeptide molecule (Figure 7 and Table 3). These ROS create an “elevated tension” of “Redox Stress” (reduction and oxidation: the damaging process of unpaired electrons attempting to re-pair to become more stable) within the islet contributing to an unstable milieu with unfolding of native protein (polypeptide) structures. This redox stress is likened to a violent thunderstorm within the islet. The unfolding of amyloidogenic amylin’s

native secondary structure to allow fibril and amyloid formation, damage to the plasma membrane via calcium channel formation, vesicle bleb formation, increased cytosolic calcium, and swelling of the intracellular organelles can be compared to lightning strikes of a thunderstorm. As nature tries to re-pair these unpaired electrons (in order to obtain a more stable electrostatic state) there will be damage to the surrounding elements (Figure 7) [23].

Each of the above toxicities are associated with the production of ROS and it is interesting to note that recently ACE inhibitors were shown to reduce the development of T2DM by 11 % in the Captopril Prevention Project (CAPPP) and again in the Heart Outcomes Prevention

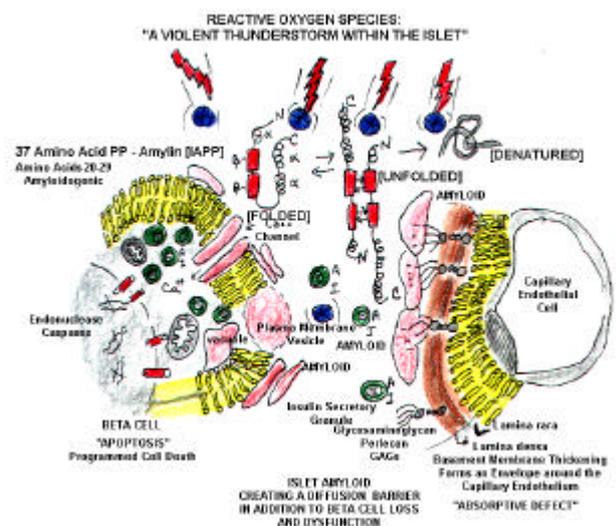


Figure 7. Redox stress within the islet.

Table 3. A-FLIGHT toxicities of insulin resistance and T2DM.

A	Amylin toxicity Ang II induces PKC	ROS ROS
F	Free fatty acid toxicity.	ROS
L	Lipotoxicity	ROS
I	Insulin toxicity (endogenous)	ROS
G	Glucotoxicity (compounds peripheral insulin resistance) Pseudohypoxia [38]	ROS plus protein kinase C (PKC)
H	Hypertension toxicity t Homocysteine	ROS ROS
T	Triglyceride toxicity	ROS

Evaluation (HOPE) study by 32%. These reductions were significant and may be related to the positive bradykinin effect (increasing nitric oxide by endothelium cells) being able to scavenge the free oxygen radical within the islet [39, 40, 41]. In addition to the redox stress within the islet there exists a local renin-angiotensin-aldosterone-system (RAAS). Tahmasebi and Vinson [42] revealed the presence of an angiotensin-1 (AT-1) receptor on the islet endothelial cells and beta cells with a monoclonal antibody for the AT-1 receptor. This makes the islet sensitive not only to the local RAAS but also, to the systemic RAAS.

Is there a mechanism for the activation of a local RAAS within the islet? Nickenig and colleagues [43] were able to show that insulin caused an up regulation of the AT-1 receptor while Cooper and McNally [44] found that amylin infusion in humans activated the RAAS with increases of renin and aldosterone. Within the islet there is an elevation of both insulin and amylin in the insulin resistant patient and early on in the type 2 diabetic patient. As a result of these mechanisms there would exist a heightened activity of the local and systemic RAAS. Therefore, as shown in the clinical trials, ACE inhibitors do have a mechanism to prevent the remodeling of the endocrine pancreas just as they do in the myocardium and the renal interstitium. There is an excellent review of the Renin-Angiotensin System in the endocrine pancreas by Per-Ola Carlson [45].

Also, pravastatin an HMG-COA reductase inhibitor was found to reduce the development of T2DM by 30% in the primary prevention trial WOSCOPS (West of Scotland Coronary Prevention Study) its exact mechanism of action is not known but it can be assumed it also reduces ROS through its anti-inflammatory, anti-oxidant mechanisms within the islet as well as reducing the substrates of LDL cholesterol and triglycerides while increasing the HDL cholesterol (one of the best natural occurring anti-oxidants and anti-inflammatory molecules) thus improving

endothelial cell function and the ability to resume normal synthesis of nitric oxide systemically [46]. We have been able to show in our laboratory that pravastatin inhibits collagen synthesis by vascular smooth muscle cells and fibroblasts with decreased fibrosis and extracellular matrix remodeling due to hyperhomocysteinemia and increased redox stress [47].

In addition, we feel that the A-FLIGHT toxicities are operating within the central nervous system as well as within the islet of the pancreas. In reference to the "Cognopathy" associated with T2DM there are now data available that statins (HMG-COA reductase inhibitors) significantly reduce the risk of developing dementia.

In separate studies both Jick H *et al.* [48] and Wolozin B *et al.* [49] were able to show the reductions in dementia in those that were on a statin. While these data are not proof that statins are causal in the reductions of cognitive decline they never the less are a very important finding.

As was pointed out earlier the ApoE 4 genotype is important to the development of AD. Antihypertensives (80% diuretics) reduced the relative risk (RR) to develop dementia from 2.2 to 0.9, and the RR to develop AD was reduced from 2.3 to 1.1 [50]. The ApoE 4 confers an increased risk of developing AD that is reduced when using primarily a diuretic antihypertensive. We can be assured that ACE inhibitors will in all probability reduce this risk as well as diuretics as they reduce the redox stress that is elevated within the central nervous system as well as within the islet.

From our pharmaceutical treatment cabinet we now have many drawers to open in our treatment of the insulin resistant type 2 diabetic patient. Earlier treatment and combination therapy to HbA1c guidelines would be the ideal treatment to halt and prevent the further deposition of islet amyloid by the amyloidogenic substrate: amylin. When we go to our treatment cabinet we now have available

metformin, alpha glucosidase inhibitors, thiazolidinediones, meglitinides, exogenous insulin, and in 2000 the combination metformin and glyburide. Nateglinide will be available in 2001 and possibly inhaled insulin. There are other forms of combination actively being pursued and the ones that are appealing are the combination of thiazolidinediones and metformin [51, 52] and the combination of thiazolidinediones and glinides [53].

If we make these changes for our patients we will be more successful in the management of many of the A-FLIGHT toxicities with one of the main focuses being on reversing the glucotoxicity and delaying the amyloid/extracellular matrix remodeling and progression of T2DM [8]. Our ultimate goal in the treatment of T2DM is to lower glucose and HbA1c levels without sustained elevations of insulin and amylin. As we achieve these goals we can slow and possibly prevent the continued laying down of islet amyloid and lessen the stress of intra- and extra-cellular amylin causing the beta cell to become dysfunctional and undergo apoptosis and to prevent the increasing absorptive defect of increasing endothelial cell basement membrane deposition (Figure 7).

Noting that this paradigm shift has virtually moved away from monotherapy with sulfonylureas, there remains one sulfonylurea that may be useful in this paradigm and that is glipizide XL with its gastrointestinal therapeutic system (GITS) mechanism there is protection from elevated post prandial amylin levels and sustained insulin/amylin levels [54].

The meglitinides (glinides) are rapidly picked up and have their action on the beta cell and then are rapidly cleared elevating insulin and amylin only in the immediate post prandial period. Nateglinide, the newest and fastest “in” and “out” glinide a D-phenylalanine amino acid derivative (a meglitinide analog) to date, acts through closure of the potassium sensitive ATPase channels of the beta cell resulting in activation of the calcium channel with

restoration of the first phase insulin secretion was approved in December of 2000 and will be launched by the time of reading this review. [55, 56, 57].

There is an acceleration of atherosclerosis associated with T2DM and extensive remodeling of the arterial vessel wall. The RAAS (originally the Renin Angiotensin Aldosterone System) acronym was created to facilitate the use of currently available medications to treat and prevent this malady. It also may be used to decrease the redox stress within the islet.

R - Reductase 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.

A - ACEi-prils. ARBS-sartans. Both inhibiting 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.

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.

S - Statins. Improving plaque stability 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 anti-oxidant effect within the islet promoting stability [8, 58, 59].

In addition to modifying and changing the treatment paradigm for the prevention and subsequent treatment of the adult T2DM patient we must take heed of the now emerging exponential growth of T2DM in our adolescent youth. We must take the necessary measures to

modify the life styles especially in those patients who have a much higher genetic and environmental risk [60]. These youths will not be immune to the development of islet amyloid and if this process starts earlier in the adolescent years (as does atherosclerosis in the Pathological Determinants of Atherosclerosis in Youth – PDAY - study) you can see it will only progress. These youths will be at a much higher risk for the development of the devastating complications (-opathies) associated with T2DM [61, 62, 63, 64, 65].

Now is the time to act at the national, as well as the local level, as amylin and amyloid deposits are already starting to accumulate and create damage and dysfunction to the beta cells within the islet endocrine pancreas. Generation D (the digital generation) and their parents may have to be re-educated in the importance of exercise and diet to stop this epidemic of T2DM in both the adult and the child/adolescent.

Conclusions

Since Opie's original description of islet amyloid we have come a long way. But we have much more to learn regarding the importance of how this amyloid tissue developed and its relation to the islet as well as systemic implications. For example, could hyperamylinemia be related to the multiple “-opathies” of T2DM ? [8].

It is possible that amyloid may be an ancient endogenous protective mechanism to protect cells (in this case the islet beta cell) and subsequently the host from the toxicity of its overproduction of amyloidogenic amylin or its precursor proamylin and their cytotoxicity. This toxic amylin polypeptide is encased like a cache of a spider (which ironically is composed of a secondary crossed beta pleated sheet structure as is silk from the silk worm) and this self made encasement allows protection of the beta cell. Unfortunately, the amyloid creates a diffusion barrier which is partially responsible

for the insulin secretory defect as well as the absorptive defect (Figure 7).

There are new therapies looking at ways of breaking up the beta pleated sheet structures appropriately termed “beta sheet breakers” [66]. However, it seems more appropriate to prevent the devastating effects of amyloid formation by preventing the abnormal processing and overproduction of amylin by decreasing glucotoxicity with the newer medications that affect the increased production by the liver (metformin) and medications that improve the peripheral uptake of insulin (thiazolidiones). To diagnose and treat earlier in the disease process and avoid the use of medications that result in excess stimulation of insulin and subsequently the amyloidogenic amylin by the islet beta cell secretory granule. With this approach we may be able to prevent or slow the remodeling within the islet (Table 2).

We are dealing with a disease of epidemic proportion in the United States. There are 16 million people diagnosed with diabetes and another 15 million undiagnosed. Ninety percent, or 14.4 million, have T2DM [67]. In India there is an epidemic as well with 30 million total [68] or 27 million with T2DM.

A review of four autopsy studies (two in the US, one in Germany, and one in Japan) revealed an average of 70% of T2DM patients have islet amyloid by light microscopy [12, 69, 70, 71].

Following this line of reasoning there would be 10 million patients in the US with amyloid positive islets and 18.9 million with amyloid positive islets in India. With these two countries alone there would be roughly 29 million people with amyloid positive diabetes.

The current problem is big and it has been growing exponentially throughout the world. Globally, amyloid within the islet is a serious and prevalent problem.

It is time to look for a model such as the feline model which parallels T2DM in humans with the development of spontaneous amyloid

formation within the islets and is almost identical in each of the associated diabetic “–opathies”. As *Felis Domesticus* is becoming more westernized (i.e. declawed, eating the high fat-high carbohydrate left overs - in addition to readily available cat chow -, leading a sedentary life style, and transitioning from being a hunter and gatherer to a literal “couch potato”) we can expect to see and probably are currently witnessing an exponential growth of T2DM in this species. This model would allow us to more readily translate the pathobiomolecular abnormalities associated with hyperamylinemia and the formation of pancreatic islet amyloid. We can expect to see a “pandemic” in feline T2DM just as in humans and this species has the potential for us to witness the changes and describe them with a direct application to their disease as well as to *Homo Sapiens*.

Using the feline model would allow us to better understand the process of amylin derived amyloid formation and the role it plays in the development of the diabetic opathies not only in the feline model but in humans [72, 73, 74, 75, 76, 77, 78, 79].

As this transformation in the treatment paradigm occurs there should be a consideration given to transform the naming of T2DM. In the 1970s we used the term adult onset diabetes, then maturity onset diabetes, then in the 1980s the term changed to non insulin dependent diabetes mellitus (NIDDM), and in the 1990s type 2 diabetes mellitus. It is time to consider using the pathologic findings in describing and naming this form of diabetes mellitus. Type 1 diabetes mellitus: “Autoimmune Diabetes” and type 2 diabetes mellitus: “Amyloid Diabetes”.

Regarding the new treatment paradigm: in order to treat earlier, now may be the time to rejuvenate the 75 gram oral glucose tolerance test. By checking both a fasting blood glucose and a two hour post prandial blood glucose we may be able to identify those who are at greater risk and be able to intervene with a resulting

decreased morbidity and mortality. A recent publication by Tuomilehto *et al.* found from the Diabetes Epidemiology Collaborative analysis Of Diagnostic criteria in Europe (DECODE) study that the two hour post glucose concentrations are better predictors of mortality than the fasting glucose alone. The two hour glucose would identify the patient with impaired glucose tolerance (IGT) (Table 2) [80].

Stabilizing the Vulnerable Islet

This article has pointed to the vulnerability of the islet and the progressive dysfunction and eventual failure of the beta cell within regarding the development of T2DM. It is time to make a shift in the treatment paradigm to earlier diagnosis and a change in the treatment modality to result in decreased glucotoxicity, insulin, and amylin levels to reduce the A-FLIGHT toxicities associated with redox stress within the islet.

The paradigm shift in the treatment of T2DM may allow us to stabilize these vulnerable islets and prevent the development of T2DM just as we have been able to stabilize the vulnerable plaque in atherosclerosis with the use of statins and angiotensin converting enzyme inhibitors. Utilizing the RAAS acronym for the prevention and treatment of arterial vessel wall remodeling and the development of T2DM will allow us to stabilize these vulnerable islets (Table 2).

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Abbreviations AD: Alzheimer's disease; AT-1: angiotensin-1; CAPPP: Captopril Prevention Project; CRP: C-reactive protein; DECODE: Diabetes Epidemiology Collaborative analysis Of Diagnostic criteria in Europe; GAGs: glycosaminoglycans; GITS: gastrointestinal therapeutic system; HbA1c: hemoglobin A glycosylated c; hIAPP: human islet amyloid polypeptide; HOPE: Heart Outcomes Prevention Evaluation; IAPP: islet amyloid polypeptide; IGT: impaired glucose tolerance; NIDDM: non insulin dependent diabetes mellitus; PC: proprotein convertase; PDAY: Pathological Determinants of Atherosclerosis in Youth; PKC: protein kinase C; RAAS: renin-angiotensin-aldosterone-system; ROS: reactive oxygen species; RR: relative risk; SAA: serum amyloid A; SAP: serum amyloid P; T2DM: type 2 diabetes mellitus; UKPDS: United Kingdom Prospective Diabetes Study; WOSCOPS: West of Scotland Coronary Prevention Study

Acknowledgements Recently, Clark A in 1996 [11], Khan SE in 1999 [81], Hoppener JWM in 2000 [82], and Westermark GT and Westermark P in 2000 [83] have published seminal review papers on this topic of islet amyloid and the role it plays in T2DM. This paper is written to honor all of those who are leaders in this area of islet amyloid and to honor our beloved patients and pets with both autoimmune and amyloid diabetes.

Correspondence

Melvin R Hayden
Department of Cardiovascular Atherosclerosis,
Metabolism and Aging
Camdenton Community Health Center
P.O. Box 1140
Highway 5 North
Camdenton, Missouri 65020
USA
Phone: +1-573.346.3019
E-mail address: mrh29@lakeozarks.net

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