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The Role of Amylin in Type 2 Diabetes

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Abstract
Type 2 diabetes is a worldwide epidemic due to many increasing risk factors such as obesity. New avenues are constantly being explored to find solutions for this impactful disease. Biochemical means are being looked into as a possible solution due to the polypeptide Amylin. An explanation for the connection between Amylin and type 2 diabetes is given, by explaining each separately and then connecting the amyloid fibril formation to type 2 diabetes. The information provided was collected through an extensive literature review.

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THE ROLE OF AMYLIN IN TYPE 2 DIABETES

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Introduction to Type 2 Diabetes:

Diabetes is a chronic disease that affects millions around the world, and is also the leading cause of kidney failure, nontraumatic lower limb amputation, and new cases of blindness among adults. Overall, it is the seventh leading cause of death and a major cause of heart disease and stroke. A disease that affects more than twenty five million people of all ages and 8.3% of the American population is one that needs to be urgently addressed from all angles possible. (National Diabetes Clearinghouse, 2011). Not only is diabetes a leading cause or underlying factor in many other life threatening illnesses, but it also is a very costly disease. Statistics released in 2007 by the National Diabetes Association estimated that the total cost of diagnosed diabetes in the United States was $174 billion, with $116 billion being spent on direct medical costs, and $58 billion being spent on indirect costs such as disability, work loss, or premature mortality (American Diabetes Association, 2011).

The media can drastically shape the public's opinion on a disease that affects so many people because people want to find out about their disease themselves and not just through their doctors. This means that they obtain their information on the disease from the large number of advertisements and articles published in the media. The public's perception of diabetes is a generally uneducated view of the disease in which people may look at it and assume the persons suffering brought it upon themselves or that it is not a serious condition. A common public misconception, which is perpetuated through the media, is that diabetes is a disease caused by overeating and lack of exercise, because the media puts so much focus on lifestyle and obesity being causative factors. The media gives double the amount of coverage to the behavioral risks of diabetes than to any other contributing factors, according to Sarah Gollust, an assistant professor at University of Minnesota School of Public Health (Lynch, 2012). Another public

misconception is that diabetes is not a serious condition and just requires the patients to be subject to finger pricks and medication for the rest of their lives.

Diabetes is divided into type 1 and type 2 diabetes mellitus based on several factors. The main distinguishing factor is whether or not the person is insulin dependent or if an insulin resistance is developed over time. While type 1 and type 2 diabetes can occur at any point in life, type 1 diabetes is usually characterized by an onset at an early age or in adolescence. The exact cause of type 1 diabetes is unknown, but it is thought to be beta cell destruction that leads to the pancreas producing no insulin for the body (Eckman, 2011).

It is a fatal issue in type 1 diabetes if the body does not produce insulin, because insulin is a hormone that is needed to convert glucose, more commonly known as sugar, into energy for the cells of the body to use to perform vital functions. Without insulin, the body enters a state of hyperglycemia, which is a high blood glucose level, because all of the glucose taken in by the body remains in the bloodstream without insulin to help the sugar enter the body's cells. This is the reason all type 1 diabetics are considered insulin-dependent because their bodies do not produce the insulin necessary for survival, so people with type 1 diabetes must supplement the insulin in their systems, in most cases through an injection or pump.

Type 1 diabetes is a rather straightforward condition where the body does not produce insulin, and, therefore, it must be supplemented for survival, but type 2 diabetes is much more complex because there are multiple ways in which it develops within the body and several different routes of treatment. Of the 8.3% of the population that has diabetes (over 25 million individuals), 90-95% of those individuals are diagnosed with type 2 diabetes. While these numbers in themselves are staggering, it is estimated that of these 25 million people, 7 million people are undiagnosed,

and on top of this number, 79 million people have been diagnosed with a condition called prediabetes (American Diabetes Association, 2011).

Prediabetes is the classification of having a slightly elevated glucose level from 100-126 mg/dL, which is higher than the recommended 80-100mg/dL, but is not high enough to be considered type 2, which is above 126mg/dL, according to the American Diabetes Association (2012). Prediabetes is becoming an increasingly prevalent diagnosis from doctors worldwide as it is becoming more necessary to diagnose prediabetes since treatment of this condition could lead to the prevention of a more serious condition. According to the Mayo Clinic, "...without intervention, prediabetes is likely to become type 2 diabetes in 10 years or less" (2012). The goal in diagnosing prediabetes is that an individual will take the correct lifestyle modification steps to bring one's blood sugar levels back to normal instead of allowing the long-term devastation that type 2 diabetes can have on the body to develop.

Type 2 diabetes differs in presentation from type 1 in that it typically has a later onset in life than type 1, but in recent years, the drastic increase in childhood obesity has also led to higher rates of prediabetes and type 2 diabetes in people under the age of twenty. As per the American Diabetes Association's statistics released in 2011, 215,000 people under the age of twenty have Type II diabetes, meaning that one in every four hundred people in this age group has diabetes. Type 2 diabetes can be asymptomatic for a very long time, which is why the numbers are so high for undiagnosed diabetes. As mentioned previously, type 2 diabetes can develop for many reasons showing a prevalence in people of certain ethnicities, in people who are obese, and in people with a strong family history of the disease. All of these characteristics are risk factors that raise an individual's chances of developing this life-altering disease. Once the American Diabetes Association accounted for the adjustment in population age differences,

the latest numbers from 2007-2009 of people diagnosed with diabetes show that the highest ethnic group over the age of twenty was non-Hispanic African Americans at 12.6%, followed closely by Hispanics at 11.8% (2012).

Diagnosis of type 2 diabetes usually occurs at a routine physical when blood work is taken and the fasting plasma glucose levels come back elevated. A fasting plasma glucose is the most common test done in a blood work up when checking blood sugar levels, but, to be diagnosed with type 2 diabetes, the test must be done after a twelve hour fast on two separate days, with numbers coming back above or equal to 126mg/dl, when normal numbers are considered to be 80-100mg/dl. Standard lab testing will allow for the normal values to be met if the fasting is done correctly, but it is very difficult to diagnose a person with diabetes from one set of values due to many outside factors that could influence test values the day blood is drawn. For this reason, it takes a minimum of two tests resulting in numbers that suggest high blood glucose to diagnose an individual with diabetes (Kaput et al. 2007).

If a person develops type 2 diabetes it can be as a result of many risk factors, including obesity or a strong family history. There is thought to be a genetic link if family members have a history of the condition, and obesity, coupled with a lack of exercise can lead to type 2 diabetes because it can cause insulin resistance. With type 2 diabetes, pathogenesis can occur in the form of two defects. Unlike type 1 diabetics, type 2 diabetics produce insulin, but the pancreas either does not produce enough insulin or the insulin cannot be used by the body as it is supposed to be used. If enough insulin is not produced or it cannot be taken up into the cells as it is supposed to be, glucose builds up in the bloodstream (Kaku, 2010). With a build up of glucose in the bloodstream due to the resistance of cell uptake of glucose, multiple body systems can be harmed, and cells cannot function properly because they are not receiving the energy they need

from the glucose. Insulin is always released in small amounts by the pancreas in order to regulate the amount of glucose being delivered to cells for energy, and when the amount of glucose in the system rises, the pancreas is triggered to release more insulin to compensate and push the increased glucose into the cells, causing the amount in the blood stream to drop. When the blood glucose levels begin to get too low, a condition known as hypoglycemia, the body begins breaking down glycogen to create more glucose, as well as signaling the body to eat in order to gain more glucose from the carbohydrates in a meal. In a type 2 diabetic, this homeostatic system is not kept in balance because either the pancreas cannot produce enough insulin to keep up with the demand or the body's cells develop an insulin resistance that strictly restrict or discontinue the uptake of glucose by the cells. Both deficiencies lead to a high blood glucose level above 126 mg/dL (DeFronzo 2004).

Just as diabetes is caused by pathophysiology within the body through issues with the pancreas creating insulin or the liver releasing glucose, there is a pathogenesis behind the complications for which diabetes is most commonly known, such as the degradation of endothelial and mesangial cells (Brownlee, 2004). The reasons these cells are so vulnerable to high blood glucose is because they are not able to control their intake of blood glucose like other cells in the body, which are able to adjust to the high levels of blood glucose and develop a resistance to allowing more blood glucose to enter cells by closing channels or transporting in less. Cells of the retina or of the nephron lack this innate ability to do adjust and resist and therefore are damaged by hyperglycemia. These repeated acute changes in the body's cellular metabolism lead to both micro and macrovascular tissue damage. Over a prolonged period of time, the cumulative changes summate and the damage becomes irreversible. Morever, when independent accelerating factors that often coincide with diabetes, such as hypertension or

hyperlipidemia, occur, these complications become increasingly significant. This degenerative path that begins with a simple elevated blood glucose level can lead to complications, such as retinopathy resulting in blindness or nephropathy resulting ultimately in kidney failure (Brownlee, 2004). Not only is it important to treat such a chronic disease to prevent the immediate harm to the body, but also to minimize the serious complications that type 2 diabetes can cause if not treated.

Treatment plans for type 2 diabetes can vary greatly, beginning with lifestyle modification and including medication, as well as insulin for the more extreme cases where there is an insulin deficiency. With lifestyle modification, type 2 diabetes can be reversed, and blood glucose levels restored to normal values, which are 80-100 mg/dL. Obesity is a big contributor to type 2 diabetes, and it is one of the biggest risk factors. Lifestyle modification will often include eating a healthier diet and exercising more often. The most important macronutrient to a diabetic's diet is carbohydrate when it comes to maintaining blood glucose levels. There are simple carbohydrates, which will raise blood glucose quickly, and there are complex carbohydrates, which will provide a slower rise in blood glucose but will keep the levels more even. Simple carbohydrates, which are necessary in a hypoglycemic state and can be utilized faster due to being digested faster include foods such as juice, candy, cookies or tablets of pure glucose. Complex carbohydrates, which should be consumed during meals to provide a steady level of blood sugar, include whole grains, starchy vegetables, and pasta. When modifying one's lifestyle in order to bring about a positive change in blood glucose levels, the goal in obese or overweight individuals is weight loss because weight loss can lead to increased glucose uptake in cells and decreased resistance to insulin. In order to improve these odds, a diet can be modified to decrease sugar, fat, and salt intake and to increase whole grain, legume, and fiber intake. The

American Diabetes Association suggests a meal management method called the Plate method, where a plate is cut in half. On one half of the plate, there are vegetables. The other half is then cut in half again, with one half being filled with potatoes, rice, or pasta, and the other half being filled with meat, poultry, and fish. Type 2 diabetics can focus on a food's glycemic index, a measure of how a carbohydrate-containing food will raise blood sugar, when choosing what to eat in each meal (American Diabetes Association, 2012). A glycemic index is based off of a reference food being glucose or white bread, and foods with a high index can be balanced with foods with a low index to create a healthy balance. Examples of foods with a high glycemic index include white bread, bagels, pretzels, and melons all of which will raise blood glucose quickly. Foods with a low glycemic index include oatmeal, most fruits, and non-starchy vegetables. Paying close attention to the glycemic index of foods in combination with carbohydrate counting is a great way for type 2 diabetics to try and control their blood glucose based on their diet. Not only does lifestyle modification ensure that a diabetic is making better food choices, but it also includes increasing the amount of physical activity received.

In December of 2010 the American College of Sports Medicine and the American Diabetes Association released a joint statement regarding exercise and type 2 diabetes. This article makes the bold and clear statement that "it is now well established that participation in regular physical activity improves blood glucose control and can prevent or delay type 2 diabetes, along with positively affecting lipids, blood pressure, cardiovascular events, mortality, and quality of life" (Colberg et al. 2010). The combination of physical activity and modest weight loss can decrease the risk of this chronic disease in high risk populations by 58%, and can be accomplished by both aerobic and resistance training. It is thought that the preferred type of exercise for a type 2 diabetic is to exercise at 50-70% of a maximal heart rate for 40-60 minutes

about five days a week. The combination of resistance training and aerobic training is best suited for blood glucose control because, " any increase in muscle mass that may result from resistance training could contribute to BG uptake without altering the muscle's intrinsic capacity to respond to insulin, whereas aerobic exercise enhances its uptake via a greater insulin action, independent of changes in muscle mass or aerobic capacity" (Colberg et al. 2010). This joint statement also went on to discuss the acute effects of exercise on glucose levels, including increased glucose uptake by muscles increased glucose transport, and increased glucose metabolism, all of which are seen during exercise. Not only is evidence given for these statements, but the same studies provide evidence that the chronic effects of exercise increase insulin binding activity at insulin receptors, cells become increasingly permeable to glucose, and increase the peripheral glucose uptake is also increased. While lifestyle modification through healthy dietary and exercise habits is the first line of defense against those diagnosed with type 2 diabetes, it is not always successful or an effective enough treatment to control the diabetes. In this case, medication can be used to try and control blood glucose levels.

Oral medication is the first line of defense when it comes to medically controlling type 2 diabetes. There are two major classes of oral medications that are seen most commonly and work on different parts of the pathophysiological pathway. The first type discussed is also the oldest type of medication called Sulfonylureas. The action of this type of medication stimulates the pancreas to release more insulin, and its advantage is speed of onset. Some examples include Glipizide and Glimepiride (MayoClinic, 2011). The other class of medication, which is by far the most commonly used oral medication for type 2 diabetes, is the Biguanides, which include the medication Metformin. Metformin is the first line of defense in oral medication for type 2 diabetics due to its affordability, drug safety, and efficacy. The mechanism of action of a

Biguanide is to inhibit the release of glucose from the liver and to increase cell's sensitivity to insulin. As far as drug safety is concerned Metformin's largest counter-indication relates to type 2 diabetics with poor renal or liver function. Depending on how severe the type 2 diabetes has become in an individual, nephropathy can be caused by type 2 diabetes, and, therefore serum creatinine levels must be checked thoroughly before prescribing Metformin (MayoClinic, 2011).

In cases where lifestyle modification and oral medication is not enough to control the diabetes, insulin may be prescribed as part of a treatment regimen for a type 2 diabetic. The insulin acts the same way in type 1 and type 2 diabetes, where it is replacing what the body cannot naturally make itself. In a type 2 diabetic, the body may still be producing insulin, but even through oral medication, it may not be producing enough to keep up with the demands of the cells in the body, so injections of the hormone must be made subcutaneously to provide the body with what it cannot produce on its own. Lifestyle modification, oral medications, and insulin are all treatment options available for type 2 diabetics, and they generally proceed in such an order. If the diabetes is caught early enough, then the hope is that exercise and healthy eating can be a sufficient treatment to stimulate positive bodily effects allowing more glucose utilization and insulin production. Regardless of the ability for these goals to be reached through a change in habits or through medication, there is no cure for diabetes. Once diabetes is diagnosed, a cure does not exist that can make one free of the chronic illness (MayoClinic 2012). Diabetes can be reversed if it is well-maintained, but it is a constant battle that will forever be fought within one's body.

The future options for treatment of type 2 diabetes are endless and are being approached from as many innovative angles as possible. The current treatment for the disease is symptom management – future treatments will be looking for a cure or at least for a way to manage this

disease, whether it be through genetics, new medications, or a biochemical approach. New advances are being made every day in the hopes of making this disease an illness of the past, because, at the rate at which it is growing, the diabetes epidemic is something that needs to be stopped in some way. One very promising lead seems to be through the investigation of a particular polypeptide, Amylin, and it's potential effects on the seventh most deadly issue faced worldwide.

Introduction to Amylin:

Amino acids are the building blocks for proteins that form the primary structure by the linkage of peptide bonds. Every amino acid contains an amino group, as well as a carboxyl group, and an R group, which is a carbon chain that uniquely identifies each amino acid. The chemical properties of the R group are what classify an amino acid. In the peptide bonds that connect amino acids, the amino group of one bonds to the carboxyl group of another, forming a peptide. A polypeptide is a long chain of amino acids linked together. The formation of a protein is derived from a single or multiple polypeptides linked together to form tertiary and quaternary structures. The primary structure is the order in which the amino acids are linked together by peptide bonds, and the secondary structure is due to the hydrogen bonding between the oxygen atom of one amino acid and the amine hydrogen of another. The tertiary structure is derived from the three dimensional structure formed when the secondary structure folds on itself, and the quaternary structure involves several polypeptides bonding together.

The peptide in question with regards to type 2 diabetes is amylin, or Islet Amyloid Polypeptide (IAPP). IAPP is a 37 residue polypeptide hormone that is secreted in conjunction with insulin from the pancreatic beta-cells in a 100:1 ratio (Brender et al. 2011). IAPP is expressed on gene 12 by one single gene copy on the short arm of the chromosome (Westermark

et al. 2011). In type 2 diabetes. amylin misfolds and causes fibril formation. Usually, amyloid fibrils are formed by soluble proteins, which assemble to form insoluble fibers that are resistant to degradation. In a healthy individual, amylin is cosecreted with insulin from the beta cells and is then excreted via the kidneys. The occurrence of islet amyloid fibril formation is less than 15% in non-diabetic patients, but is present in over 90% of diabetic subjects (Jaikaran, 2001). Amyloid is only seen in type 2 diabetics, because, in type 1 diabetics, the IAPP source is removed due to the destruction of the beta cells. Because insulin and IAPP are cosecreted, an increased level of human Islet Amyloid Polypeptide (hIAPP) which occurs in a state of insulin resistance, where insulin secretion increases to compensate can initiate the fibril formation. When IAPP is stored in the secretory granules of the beta-cells at about one to four millimolar concentration, which is about a thousand times the amount that is necessary to form amyloid fibrils.

There are certain characteristics of amylin, as well as amino acid residues in the 37 amino acid peptide, that are very important in the process of fibrillar formation of amylin. One characteristic IAPP shares with other peptides in the calcitonin family is the inclusion of a disulfide bridge between residues 2 and 7 and an amidated COOH terminus, which are modifications made after translation that are important to the biological function of these peptides in the calcitonin family (Westermark et al. 2011). In this same article, "Islet Amyloid Polypeptide, Islet Amyloid, and Diabetes Mellitus", Westermark and colleagues described the tertiary structure of IAPP;

> "Both CD and NMR studies have shown that the peptide forms an at least transient amphipathic helix in the NH2-terminal region, except for the very NH2- terminal part, which forms a rigid ring structure, resulting from the disulfide bridge between residues 2 and 7. In solution, the helix spans residues 5–23. The COOH-terminal part of the

molecule is unstructured. The helical part is believed to be important in receptor binding and may also be deeply involved in the pathological transformation to amyloid fibrils" (2011).

Proline substitutions in the amino acid residue region on IAPP from 20-29 are involved in rat islet amyloid polypeptide (hIAPP) and rats do not develop diabetes (Jaikaran, 2001). This indicates the importance of this region in forming amyloid. There is also a second amyloidogenic region, hIAPP 30-37 which when placed in a medium formed fibrils, as well as a third region from 8-20 that is under investigation. These three proline residues are seen in rat IAPP, but not in human IAPP and the presence of these proline residues is thought to prevent hIAPP from becoming amyloidogenic. The aggregation of hIAPP is completed within a few hours depending on other factors, such as the presence of lipids, and this aggregation is significantly faster than those of most other amyloidogenic peptides. According to Brender and colleagues, "Amyloids form through a nucleation-dependent process leading to characteristic sigmoidal type kinetics; in which amyloid formation is minimal (the lag-phase) until a critical concentration of nuclei is reached, at which point amyloid growth proceeds explosively" (2011). During the formation of stable fibrils, nucleation must occur, and the nucleus is an oligomeric hIAPP species that can serve as the template for the fibrillar version. Initially, an alpha-helical structure is adopted before transforming into a beta-sheet structure, and it is thought that the oligomers can exhibit an alpha-helical structure. During the formation of the amyloid fibrils, a conformational change takes place, in which the structure changes from a random coil to a mixture of alpha-helical and beta-sheet structure (Khemtemourian et al. 2008).

The cardinal finding in type 2 diabetics as far as pathogenesis in the islet cells is the finding of these amyloid fibrils. Characteristics of the amyloid fibrils found in these islets

include them being unbranched, thin, and of an indefinite length. The monomers are arranged in a beta-sheet perpendicular to the fibril axis. The nucleation process of amyloid formation can be broken down into three phases, the first of which is called the lag phase. Here, the nucleation of these monomeric peptides occurs. The second phase is known as the elongation phase, where the critical amount of nucleation can occur, and then in quick fashion propagates the formation of the fibril. As discussed above, once the nucleation occurs, the formation of the fibrils is a very quick process in reference to IAPP, whereas other amyloid fibril formation is typically done at a slower rate. Once the elongation phase has completed, the third phase in in vitro systems is the plateau stage, where the fibril has reached a constant mass and is considered to be in its steady state (Westermark et al. 2011). The interesting revelation about amyloid formation is that the mature fiber does not have any significant cell toxicity. While the buildup of amyloid fibrils in the islet cells is an indicator of type 2 diabetes, as well as other types of amyloid fibril build up in other organ systems being large enough masses to cause severe disease, the mature fibrils themselves are relatively inactive. What seems to be causing the cytotoxicity is the intermediates from IAPP to amyloid fibrils, namely the oligomer transition states. "Subsequent reports have underlined that it is small, oligomeric IAPP aggregates and not fibrils that constitute the toxic species," according to Westermark and associates in his article published by the American Physiological Society in 2011.

Oligomers are ill-defined and hard to study because they can only be studied after they have formed, and they have been exclusively studied in vitro, with the suggestion that they are inserted into cell membranes. It has been suggested that the cytotoxicity arises from the damage these oligomers cause, whether it be through the formation of pores in the membrane or through nonspecific membrane disruption. The methods by which these oligomers cause cytotoxicity are

still topics of investigation. In Westermark's article, an explanation for the cytotoxicity is explained as the following process,

> "In a proposed model on IAPP cytotoxicity, based on findings when IAPP was absorbed onto or inserted into a lipid membrane, the 19 NH2-terminal amino acid residues are inserted in the membrane. Insertion in this way leaves the amyloidogenic segment of residues 20 –29 free to aggregate, and fibril growth will force the membrane to rupture" (2011).

Now the question is turned away from the issue of mature fibril formation and is becoming more of a question of the oligomeric intermediates associated with beta cell death. The key to connecting the role of amyloid fibril formation and type 2 diabetes lies in understanding the mechanism behind the cytotoxicity of the intermediates. If the mechanism of cytotoxicity can be determined, whether it be through damaging the membrane or some other form, then it gives a starting point to determine how to prevent the damage causing the cell toxicity. Research shows that the aggregation of IAPP oligomers plays a crucial role in the progression of beta cell death in transplanted islet cells, leading to support for a similar mechanism in type 2 diabetes. Such research results that could lead to an explanation of cell toxicity in the beta cells and could also open doors for the possibilities of new treatments for type 2 diabetes.

Connecting Amylin and Type 2 Diabetes

There are many different factors that allow the assumption to be made that the fibrillar transformation of the 37 amino acid residue, amylin, into a mature amyloid fibril, which occurs in a process producing many oligomeric intermediates, can be linked at the biochemical level as part of the cause of beta-cell death that can lead to type 2 diabetes. Proof of such a statement has been shown through amyloid formation in transplanted pancreatic islet cells. Experimental islet

cell transplantation was done, in which human islet cells, once they became available, were made diabetic by injection and were then used in studies testing the effects of hyperglycemia on human beta cell function. The following quote describes the importance of the human islet grafts becoming available and their effect on the research of amyloid fibrils in diabetes.

"Keeping in mind the difference in the IAPP amino acid sequence that prevents amyloid formation in rodent islets, the availability of human islet specimens in an in vivo setting of this kind has paved the way for experimental studies of the presence of fibrillogenic IAPP and accompanying amyloid formation in human islets" (Westermark et al. 2011).

With the human islets it was found that the alpha cells remained intact, while the beta cells decreased in number and amyloid fibrils were found densely packed intracellularly with their plasma IAPP levels being increased more than five times that of the baseline value. This data in human islets differed from rat islets, which showed only very sparse amyloid fibrillar formation, but that was to be expected due to the differences in the amylin sequence that prevent fibril formation in the rodent IAPP amino acid sequence. The results of such studies of transplanting the pancreatic islet cells proved a firm connection between amyloid fibrils and the destruction of beta cells that can lead to type 2 diabetes:

> "Furthermore, recent studies of human IAPP transgenic islets or control islets grafted into streptozotocin-diabetic mice showed that hyperglycemia recurred only in mice that had received transgenic islets. Amyloid deposition occurred prior to the recurrence of hyperglycemia, and was accompanied by increased rates of beta-cell apoptosis and decreased beta-cell replication. The accumulation of amyloid correlated with the loss of beta-cells "(Westermark et al. 2011).

Type 2 diabetes is "characterized by a slowly progressive degeneration of islet beta-cells, resulting in a fall of insulin secretion and decreased insulin action on peripheral tissues" (Miklossy et al, 2010). This same study, published by Miklossy and colleagues in 2010, demonstrates the deposition of amyloid material as a major pathological factor in type 2 diabetes. The facts indicate that amyloid deposits are seen in over 95% of type 2 diabetics and that the severity of the disease positively correlates with the extent of islet amyloid deposits. The presence of clinically silent and moderate amyloid deposits can possibly correspond with early, preclinical stages of type 2 diabetes. The correlation between amyloid deposits and type 2 diabetes provides a window of opportunity for an early detection of the disease through this post-mortem research.

Beta cell death is one of the causes behind type 2 diabetes because, when the beta cells die, insulin is not being released to keep blood glucose levels within the normal range of 80-100 mg/dL. Without the beta cells from the pancreas to synthesize and release insulin, hyperglycemia will occur. As previously stated, IAPP fibrils are located at the cellular membrane of the Islet of Langerhans, and the presence of these fibrils leads to alterations in the membrane morphology. One hypothesis states that the membrane could be the target of the cytotoxicity of these fibrils, causing the death of the beta cells that produce insulin. Type 2 diabetes is associated with increased insulin resistance, and, since IAPP is coproduced and cosecreted with insulin, an increase in the level of IAPP in human cells could initiate IAPP fibril formation. This would produce an altered ratio of insulin to IAPP, which is observed in diabetic patients and could lead to a decrease in the inhibitory effect of insulin on IAPP amyloid fibril formation. In a healthy individual, amyloid is not observed, in part because the fibril formation is typically inhibited by insulin. Insulin should be produced in 50-100 molar excess of IAPP, and this is a sufficient

amount for it to suppress amyloid formation in healthy individuals (Hebda and Miranker, 2009). In an article, "Atomic Structures of IAPP Fusions Suggest a Mechanism for Fibrillation and the Role of Insulin in the Process", published in 2009, offers evidence through a fusion crystal structure that suggests, " Insulin binds to IAPP in the same way that an IAPP monomer binds to the second monomer of the dimer...These molecules rotate to bury more surface area than in the IAPP dimer structure, suggesting the possibility of a tighter interaction between IAPP and insulin than between two IAPP molecules" (Wiltzius et al.).One hypothesis is that an increase in IAPP-membrane interactions could lead to the development of type 2 diabetes (Khemtemourian, 2008).

One of the most widely investigated hypotheses that approaches the connection between type 2 diabetes and IAPP suggests that the damage caused by its interaction with the cell's membranes. Observations have shown that phospholipid membranes promote the aggregation of human IAPP, and that in the presence of phospholipids there is a reduction in lag time, causing fibril formation to occur earlier. Cell membranes may accelerate hIAPP fibril formation by enhancing nucleation, and formation of these fibrils is markedly accelerated in the presence of membranes containing negatively charged lipids. The time difference is substantial, with fibril formation occurring within a few minutes in the presence of these negatively charged membranes as opposed to within a few hours in the absence of such membranes (Khemtemourian, 2008). It has become clear that the cytotoxicity of these fibrils is derived from their intermediate states rather than from their mature fibrils (Hebda and Miranker, 2009). The review article published by Khemtemourian and colleagues discussed studies performed to determine the conformation of hIAPP that interacts with model membranes. The model membranes were composed of a neutral phospholipid and a negatively charged phospholipid and when hIAPP, when incubated with the model membrane for forty minutes, made changes to the

beta sheet conformation, which is the characteristic of fibril formation as already discussed. Using microscopy techniques, it was found that hIAPP morphology was connected to channel-like behavior indicating that the oligomeric (fewer than 5 monomers linked together) hIAPP pores could become part of the membrane, changing the barrier properties of such membranes. This experimental diabetes research took a step further to look at the mechanism of cytotoxicity, stating that the membrane is the target of the cytotoxic IAPP leading to the death of the insulin-producing beta cells. The mature hIAPP fibrils were found to be less cytotoxic and did not cause the membrane damage that the oligomeric form of hIAPP did. This review by Khemtemourian discusses the view that "IAPP-induced membrane damage, and concomitant beta-cell death, is caused by cytotoxic hIAPP oligomers" (2008). Surprisingly, once the oligomers matured into fibrils the pores formed in the membrane disappear and damage decreases. Yet the oligomeric form of hIAPP can carry out a general destabilizing effect when applied outside or inside a cell. If these oligomers can reach the endoplasmic reticulum or mitochondria within a cell endoplasmic reticulum stress occurs leading to the apoptosis of the beta cells (Khemtemourian et al, 2008). It is able to be seen that the mature amyloid fibrils would be less destructive because they are stable, proteinaceous structures. In Hebda and Miranker's study of type 2 diabetes and the role that catalysis and toxicity of amyloid plays on the lipid bilayer, the statistic was presented that over 90% of type 2 diabetics have evidence of amyloid deposition, but the question arises about the other 10%. The remaining percentage of patients are what led to the theory that the intermediates of amylin may play a role, because a protein can be ascribed to a specific disease as a causal factor, but it does not have to be the mature fibrillar form that causes disease all the time. Similar studies were done using IAPP-membrane interactions with rodents and mouse transgenic lines of hIAPP in five different studies conducted by three different

groups. The rodents developed a loss of up to 60% of their beta cells correlating with hyperglycemia and amyloid deposition. The proof that intermediates of amyloid play a more cytotoxic role in type 2 diabetes is that in rodent studies done using the introduction of a single amyloidogenic peptide into nontransgenic rodent models, an increase was observed in beta-cell death without the subsequent amyloid depositions. This provides evidence that not plaques of IAPP fibrils depositions but more likely the intermediates that form such plaques contribute to the origin of beta cell toxicity (Hebda and Miranker, 2009). This study, as well as Khemtemourian's, points to the possibility of endoplasmic reticulum stress as a possible origin for amyloid induced toxicity. The fact remains that a definite answer is yet to be discovered and, "For IAPP, toxicity could be the result of membrane disruption, at any point along the secretory pathway, from ER to Golgi, granule, or plasma membrane" (Hebda and Miranker, 2009).

Through newer technologies that are constantly evolving and being used to test hypotheses such as the ones suggested above, advancements have been made to prove the IAPP-membrane interaction hypothesis. A study published results in the Journal of Molecular Biology in 2012, relying on fluorescence microscopy leakage assays to reveal the membrane-permeabilizing effect of IAPP. In order to detect the permeabilization of vesicles that should occur during aggregation and fibril formation, IAPP was labeled at the C-terminus with a lysine residue, and when fluorescence microscopy was used, pure IAPP was seen in the interface of the lipid vesicles five minutes after it's addition. Within the next few minutes IAPP amounts increased leading to permeabilization and leakage of the membrane. Even after forty minutes of aggregation, IAPP aggregation of vesicle-like shape was still detectable, suggesting that some of the IAPP was incorporated into the growing peptide aggregates (Seeliger et al. 2012). The final conclusions of this study led to the postulation of a multiple step mechanism where the insertion

of the positively charged N terminus of IAPP into the anionic membrane was followed by a rapid transition from a disordered alpha-helical to a beta-sheet confirmation. Virtually all of the evidence lends support to the IAPP-membrane interaction hypothesis where, "IAPP showed significant cytotoxicity of IAPP oligomers, which may permeabilize the β-cell membrane as shown by a fluorescence microscopy leakage assay on isolated β-cells in the present study" (Seeliger et al. 2012).

IAPP is believed to be a major factor in the transition and pathology from early stages of type 2 diabetes to the later stages of the disease. A study, Membrane Disruption and Early Events in the Aggregation of the Diabetes Related Peptide IAPP from a Molecular Prospective states that,

> "Using variants of IAPP that are combinations of toxic or non-toxic and amyloidogenic or nonamyloidogenic forms, we have shown that formation of amyloid fibers is a sufficient but not necessary condition for the disruption of β-cells. Instead, the ability to induce membrane disruption in model membranes appears to be related to the peptide's ability to stabilize curvature in the membrane, which in turn is related to the depth of penetration in the membrane" (Brender, Salamekh, and Ramamoorthy 2011).

This is yet another avenue from which to approach the hypothesis of IAPP-membrane interaction through the intermediates of IAPP as a possible causal factor in type 2 diabetes. A unique characteristic of IAPP that is not common to other amylodogenic proteins is its transient oligomeric state that quickly converts to mature amyloid fibril. Once again, the question is raised as to the origin of the cytotoxicity, with two proposed hypotheses being endoplasmic reticulum stress due to the accumulation of misfolded proteins or the formation of reactive oxygen species due to metal complexation. This study presents two theories for the mechanism of membrane

disruption with the first being the pore theory previously discussed, in which the oligomers form pores in the membrane, causing membrane damage. There is support for this theory through fluorescence microscopy, as well as data collected by adding low concentrations of hIAPP to membranes at low salt concentrations, resulting in a fluctuation occurring in membrane concentration occurring that is reflective of the opening and closing of individual channels. The second proposal is that of a "detergent-like mechanism with mosaic-like opening and closing of transient defects within the membrane that is supported by AFM studies, which show macroscale defects in the lipid bilayer upon prolonged exposure to hIAPP" (Brender, Salamekh, and Ramamoorthy 2011). While this was investigated in the trial, it was thought to be less likely because permeabilization by hIAPP is strongly selective for smaller molecules, which weakens the case for the large-scale destruction to membranes caused by the proposed detergent-like effects.

One of the previously mentioned hypotheses proposed for the origin of the cytotoxicity was through oxygen radicals formed through the synthetic human amylin peptide, which can generate hydrogen peroxide during incubation in vitro. This study allowed for the employment of a specific spectrophometric technique to check for carbonyl group formation, which gives evidence of peptide damage due to the presence of reactive oxygen species in vitro (Masad et al. 2011). The formation of the hydroxyl radical, which was formed and proven by spectroscopy is an extremely reactive oxygen species that results in local oxidation, and, therefore damage, of the peptide. This study provided clear evidence for oxidative damage to human amylin peptide due to the detection of carbonyl groups, and

"The formation of reactive oxygen species in the presence of human amylin peptide could hold the key to a better understanding of the damaging consequences of amyloid formation within the pancreatic islets of patients with type 2 diabetes. Copper- mediated oxidation (and H2O2 generation) could be an important step in the processes leading to islet β-cell degeneration and progression of type 2 diabetes." (Masad et al. 2011).

While there have been suggestions of IAPP and amyloid being linked to type 2 diabetes and other amyloidogenic diseases, like Alzheimer's and Parkinson's disease, it was not until recent times that evidence was presented to prove such a link between amylin and type 2 diabetes. Of particular interest is the fact that while it was originally assumed that the amyloid fibril deposits are what linked type 2 diabetes to the polypeptide, it is actually caused by its intermediates. Most evidence points toward an IAPP-membrane cytotoxicity that derives from the oligomers of IAPP instead of the mature fibril. There are still questions left unanswered about the origin of the cytotoxicity of the IAPP intermediates, but cases can be made for endoplasmic reticulum stress due to the build-up of misfolded proteins, as well as for reactive oxygen radicals that may be formed. There is enough technology to support hypotheses presented from years ago based on theory, and with continuing technological advances, more answers will continue to be found. Even with the answers so far discovered, ways to prevent membrane destruction are currently in the works as well as using the correlation between amyloid deposits and type 2 diabetes as a detection tool while the disease is still clinically silent, but present in the early stages of pathological type 2 pre diabetes. Through science, answers are being found and the next step is to use such answers for early detection or in order to prevent the mechanisms that lead to membrane destruction, cytotoxicity, and diseases like type 2 diabetes.

The Future:

All of the questions that have continued to be answered about the integration of IAPP and type 2 diabetes have led to possibilities for the possible prevention of the disease. It has been shown that the major decline of glucose tolerance can be directly attributed to loss of beta cell function and eventually beta cell death (Kahn, 2003). While beta cell dysfunction is what leads to hyperglycemia and type 2 diabetes, the formation of amyloid fibrils is also directly increased with the death of these beta-cells. Therefore, if there is a distinguishable method by which to reduce the amount of amyloid fibrils that are forming, or more specifically, the oligomer transition state that leads to the formation of these mature fibrils, it may be possible to improve glucose tolerance and reduce type 2 diabetes as well.

One possibility in the improvement of glucose tolerance in type 2 diabetics is derived from the thought of amylin agonists. Since amylin is cosecreted with insulin from the Islet of Langerhans cells and it inhibits glucagon secretion, it is thought that the replacement of this peptide hormone could lead to improved glycemic control in some individuals with type 2 diabetes (Schmitz, Brock, & Rungby 2004). The article "Amylin Agonists: A Novel Approach in the Treatment of Diabetes", published by Schmitz, Brock, and Rungby, also recognizes the point that increased amylin presence can also lead to fibril formation, which subsequently leads to beta-cell death. Due to this large issue in a seemingly easy solution to the issue of hyperglycemia, a stable analog, pramlintide, has pharmacokinetic actions and pharmacodynamic properties of amylin. The stability of this analog offers beneficial uses because it will not enhance fibrillar formation, but it could improve the glycemic tolerance of individuals. It has been tested in clinical trials of over 5,000 insulin-treated patients, and 250 patients for greater than or equal to the time period of two years (2004).

The amylin analog, pramlintide, differs from amylin in that three proline residues are substituted at positions 25, 28, and 29 in the amino acid chain, or the primary structure. These substitutions make pramlintide, "stable, soluble, nonaggregating, and nonadhesive and it has been shown to exhibit beneficial actions similar to those of native amylin" (Schmitz, Brock, & Rungby, 2004). The rationale behind replacing amylin with pramlintide in humans is that it mainly exerts its effects in the postprandial state, or in the fed state. It does so by inhibiting excessive glucagon secretion and delaying gastric emptying which gives the body a longer time to process the glucose that is put into the system during a meal. A known effect of the amylin analog is it's effect on the satiety factor of obese type 2 diabetics. A 52 week study was conducted by Schmitz, Brock and Rungby on 656 type 2 diabetics who were treated with insulin. The subjects in the study were dosed with pramlintide twice daily in two different dosage groups through an injection of 90 and 120 micrograms respectively. In the group dosed with 120 micrograms, a 0.62% reduction was seen in the HbA1c levels. Also, in keeping with the positive effects on satiety in the obese type 2 diabetics, a loss of 1.4 kg was exhibited in the pramlintide groups versus a gain of 0.7 kg in the placebo group. In the pramlintide group, the daily insulin dose was significantly reduced as well (Schmitz, Brock, & Rungby 2004). The benefits exhibited in several, large-scale phase III trials of over 3,000 type 1 and 2 diabetics using the synthetic drug pramlintide include decreased HbA1c levels, weight loss, and no increase in hypoglycemic episodes. In the type 2 diabetics a sustained weight loss over a one year period was also seen.

Another article released in 2011, "Management of Type 2 Diabetes: New and Future Developments in Treatment", discusses drugs targeting beta cell dysfunction as a therapeutic class of prevention. It also discussed pramlintide as a drug that targets the pancreas and acts

directly or indirectly on beta cells, secreting insulin, C-peptide, and amylin. The amylin analogue, pramlintide, was included in a table of available drugs that lower blood glucose, and was put on the market in 2005. It was cited for its route through subcutaneous injection regarding the advantages of weight loss, and its disadvantages of only being used with insulin (Tahrani et al.).

While there is an amylin analogue currently on the market, another form of therapeutic means that is being explored includes its inhibitors.The study conducted by Hebda and Miranker in 2009 states,

> "The practical consequences of our current biophysical understanding of amyloid toxicity are twofold. First, therapeutics must prevent accumulation of toxic intermediate species...Second, a mechanistically targeted therapeutic need only make modest alterations to the levels and localization of amyloidogenic species"."

This study presents the point that targeting fibers themselves will not solve the problem, but could indeed worsen it by causing a build up of amylin intermediates which are the toxic species.

> "Suitable targets can include stability of the helical state, helix-helix interactions, protein-membrane adsorption, and the interconversion of adsorbed states from lateral to transmembrane orientations...Stabilization of a helical state or a reduction in helix- helix interaction energies can be expected to accelerate or alternatively inhibit subsequent amyloid conversion depending on the balance of energy. For IAPP, the capacity of hIAPP versus rat IAPP to assemble into a membrane- permeabilizing state rests in part on the energy of nucleating a membrane-bound helical aggregated state" (Hebda and Miranker 2009).

This suggests that a therapeutic strategy should be designed to include molecules that displace hIAPP from the membrane surface, because it may work synergistically with endogenous insulin.

A large class of inhibitors that is under development focuses on decreasing the amount of fibril formation, but as previously stated, this is not necessarily beneficial because the information shows that the known oligomer or other transition states are causing the problem and not the mature fibril. These inhibitors only decrease the formation of the fibril, not the oligomer, so they may not be particularly useful according to a review done in 2013 by Ping Cao and associates that was published on the behalf of Federal of European Biochemical Studies. This review explored another class of inhibitors that may prove to be more efficient because they interact with the monomer or very early-formed oligomers in a way that could then prevent the build up of the toxic species of amylin. An example of such an inhibitor is a biologically active flavanol in green tea, Epigallocatechin 3-Gallate (EGCG). It has been shown to bind to unaggregated polypeptides and has been shown to redirect the formation pathway of amylin to instead form nontoxic oligomers instead, meaning that EGCG can inhibit hIAPP amyloid formation, as well as protect against hIAPP induced toxicity (Cao et al. 2013). The mechanisms for inhibitors, such as EGCG, are not yet known and are still debated, but the action is decisive. The article states, "One possibility is that the compound interacts with the protein backbone and also makes non-specific hydrophobic interactions with protein side-chains"(Cao et al. 2013). While EGCG seems to be the inhibitor that could be the most effective at the moment, others are also under investigation;

> "Several rationally designed polypeptide inhibitors have been reported to inhibit hIAPP amyloid formation and toxicity. For example, certain single proline mutations in the 20–

> 29 region convert hIAPP into a potent amyloid inhibitor [82,83] and a double N-methylated variant of hIAPP has been shown to be a very effective inhibitor of amyloid formation and hIAPP cytotoxicity" (Cao et al, 2013).

It is thought that the inhibitors mentioned above work by targeting the helical oligomers, but just as EGCG, no mechanism has yet to be determined. The conclusion of this article was stated clearly and concisely by explaining that, while these findings are groundbreaking and could provide the world with solutions to type 2 diabetes, there is still research and work to be done. Some of the questions left to be answered include defining the nature of toxic species and identifying the, initiation site or sites of amyloid formation in vivo.

Conclusion:

Type 2 diabetes is a worldwide pandemic that will continue to grow and, through increased insulin resistance and decreased insulin uptake, high blood glucose or hyperglycemia, is increasingly hard to combat. Damaged beta cells and eventually beta cell death cause the pancreas to produce insufficient amounts of insulin, a hormone that helps keep blood glucose in the homeostatic range by lowering the amount in the blood when it is too high. Insulin does so by aiding in the uptake of blood glucose by cells. Without insulin, this blood glucose is left in the bloodstream and the cells cannot receive the required energy given to them by glucose, which is broken down and used by the cell. Beta cell damage and death are large parts of the pathogenesis in type 2 diabetes, and an investigation into what causes these cells to be damaged and eventually die is of great interest in trying to combat diabetes. Risk factors that can lead to diabetes include a genetic predisposition, ethnicity, and obesity, one of the largest risk factors. There are several modes of treatment currently prescribed for type 2 diabetics, the first of which includes a lifestyle modification to a healthy diet and regular exercise. It is thought that losing

weight and regular exercise can improve insulin resistance as well as insulin uptake, but for some patients these steps will not be enough to correct their hyperglycemia. If lifestyle modification is not enough, an oral agent is prescribed with one of two major mechanisms of action: either the pancreas is stimulated to produce more insulin or the liver is regulated to produce less glucose. The most popular and affordable oral agent on the market currently is Metformin. If an oral agent still is not enough to keep a patient's diabetes under control, insulin injections can be an added to supplement the body with what it can no longer produce on it's own. While all of these modifications to a person's lifestyle are made in order to combat type 2 diabetes, the biochemical world has begun to approach the subject from a molecular basis as a way to prevent the worsening of diabetes, provide early detection, or possibly one day be able to prevent its onset.

One of the avenues opened through science is that of discerning a strategy to prevent beta cell damage. Human islet amyloid polypeptide, also known as amylin, is a peptide hormone cosecreted from the islet cells with insulin. It has been seen in type 2 diabetics that this polypeptide undergoes a transformation into a mature fibrillar state, causing amyloid depositions intracellularly in the beta cells. It has now been determined that the mature state of the amyloid fibrils is not the cause of cell toxicity in the case of type 2 diabetes, but rather its oligomeric intermediates cause the cytotoxicity to occur at the beta cells. These oligomeric intermediates of amyloid fibrils have not yet been determined to have one specific mechanism of action for causing the cytotoxicity, but several theories of cell toxicity have been proposed including beta cell membrane disruption, endoplasmic reticulum stress, and oxygen radical formation. There is also a large possibility that a combination of such cytotoxic events caused by the aggregation of amyloid fibrils is what causes the damage to beta cells. The mechanism of cell toxicity due to hIAPP fibril deposition is one of the biggest questions left to answer, but even with the research

done to date, possible solutions to such a molecular level problem have been developed to aid in solving the large scale problem, type 2 diabetes.

Discoveries made regarding IAPP aggregation into mature fibrils via cytotoxic, oligomeric intermediates are on the rise and help us understand a pressing issue considering the exponential growth of individuals developing type 2 diabetes. Such answers to this problem include amylin agonists and polypeptide inhibitors. Through therapeutic solutions, such as pramlintide, a drug put on the market in 2005 to lower glucose by acting as an amylin agonist, or Epigallocatechin 3-Gallate (EGCG), produces polypeptide inhibitors that reduce the rate of fibrillar formation and hIAPP cytotoxicity such as the scientific community is slowly working its way towards answers. While there are many questions still left to be answered, there has been substantial progress over the last three decades since the discovery of amylin in 1987. This progress has led to possible answers not only to what causes amyloid fibril depositions but also to ways of preventing the healthy polypeptide from aggregating into such a harmful fibril and, thereby protecting the integrity of the beta cells of the pancreas.

Approaching type 2 diabetes from angles other than just those of lifestyle modification and medication is crucial in the race to find a way to cease the exponential growth of this debilitating disease. By using knowledge on the scientific, biochemical, and molecular levels the science community is providing another route of therapy for type 2 diabetes. It is known that beta cell death is one of the major causes of the significant decrease in the production of insulin in type 2 diabetics. The scientific community has provided an avenue of research that allows for the possibility of preserving these beta cells by understanding the mechanisms occurring at a molecular level involving amylin, which causes their death and subsequent inability to produce insulin. In a way, using the science behind the disease to prevent it from occurring can be looked

at as a complex version of preventative medicine that can aid in halting the seemingly unstoppable growth of the seventh leading cause of death in the United States, diabetes. Just as the production of amyloid fibrils increases explosively after a certain level of nucleation is reached, causing increased beta cell death, the level of obesity and unhealthy living has resulted in an explosion in the number of individuals affected by type 2 diabetes. In much the same way that amylin agonists or peptide inhibitors can prevent the molecular explosion of fibrils, continuing research at this molecular level can provide a preventative medicine approach to the disease that already affects over twentyfive million Americans. The connection between lifestyle risk factors and type 2 diabetes is as apparent as the connection between the transition of amylin into deposition of amyloid fibrils and the death of beta cells. In the same way that society intends to decrease an unhealthy lifestyles to prevent type 2 diabetes, the scientific community looks to decrease the molecular mechanisms that lead to type 2 diabetes.

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