Chronic Complications of Diabetes Mellitus
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2013

Objectives:

1. To understand the proposed mechanisms by which the Metabolic Syndrome and hyperglycemia cause the complications of Diabetes Mellitus.
2. To understand microvascular complications of Diabetes Mellitus.
3. To understand macrovascular complications of Diabetes Mellitus.

The hallmark of diabetes mellitus (DM) is an elevation of the blood glucose concentration. The blood glucose is elevated in both Type 1 and Type 2 DM and both types of diabetes are associated with many of the same complications. However, patients with Type 2 DM usually also have significant insulin resistance (hyperinsulinemia is frequently present) and are generally older. Conversely, patients with Type 1 DM are prone to have episodes of ketoacidosis (DKA), which may have long term effects on a variety of organs. These factors must be considered when comparing the prevalence and severity of the chronic complications in these two types of diabetes.

The three organs most commonly and dramatically affected by diabetes are the eyes (retinopathy), nerves (neuropathy), and kidneys (nephropathy) (the "triopathy"). However, it must be remembered that diabetes can damage almost any tissue in the body and the most likely cause of death of a diabetic patient is atherosclerotic vascular disease. Prior to the age of 40, the most common diabetes-related cause of death is renal failure but only 10% of Type 1 DM patients die during this period of time. After 40 years of age, atherosclerosis rapidly passes renal failure as the single most common cause of death.

For many diabetic patients, dying is frequently easier than living. This is because of the morbidity caused by the "triopathy" and atherosclerosis can be extremely devastating without actually causing death. Patients, therefore, can live for many years in a very debilitated state. This morbidity includes blindness, amputations, severe orthostatic hypotension, nausea, vomiting, diarrhea, constipation, loss of sensation in all extremities, impotence, recurrent infections, stroke, heart attack, congestive heart failure, and severe pain in almost any part of their body. Because of this multitude of complications, virtually all physicians will encounter patients with diabetes. It is, therefore, vital that physicians learn how to prevent or treat these many complications.

There are, at least, six metabolic abnormalities that appear to be major factors in the development of the chronic complications of diabetes and most are directly related to the elevated blood glucose, free fatty acid concentrations, and/or pro-inflammatory cytokines:

1) Increased metabolism (oxidation) of glucose and free fatty acids which produces mitochondrial "reactive oxygen species" (ROS) such as super oxide
2) A deficiency of myo-inositol leading to abnormal phosphoinositide metabolism
3) Increased metabolism of glucose through the sorbitol pathway

4) Non-enzymatic glycosylation of proteins, RNA, and DNA (covalent attachment of carbohydrate molecules to proteins and nucleic acids)
5) Abnormal extra-cellular matrix, especially reductions in heparan sulfate with an increase in collagen and fibronectin
6) In **Metabolic Syndrome**, there is the added impact of adipose tissue-derived compounds such as: leptin, adiponectin, TNF-alpha, angiotensin II, PAI-1, free fatty acids, and many more.

The development of complications in patients with insulin-resistance (Metabolic Syndrome) begins long before hyperglycemia develops. The development of the Metabolic Syndrome usually requires a combination of genetic and environmental factors. Insulin resistance and impaired insulin secretion clearly have a major genetic component but the specific genes involved have not been fully characterized. The major environmental factors are the development of obesity and reduced exercise. Fat tissue, specifically **visceral fat (in mesentery and omentum)**, secrete excessive amounts of free fatty acids (FFAs), leptin, tumor necrosis factor-alpha (TNFa), angiotensin II (AT-II), and plasminogen activator inhibitor-1 (PAI-1), while the secretion of adiponectin is reduced. Various tissues take up the FFAs where they are oxidized, stored as triglycerides, or converted to prostaglandins. The excessive oxidation of glucose and FFAs via the mitochondrial electron transport chain generates the reactive oxygen species (ROS) "super oxide" (SO), a free radical that oxidizes the cysteine amino acids in a variety of proteins. One of these proteins is RAS which, when oxidized, will activate serine-threonine kinases such as: protein kinase C, p38 MAPK, and JNK/SAPK. These free radicals themselves can oxidize and damage many cellular macromolecules, including proteins, fatty acids, DNA, and RNA.

Super oxide can be generated via coenzyme Q10 (CoQ10), an important electron transporter in the mitochondria. Normally, CoQ10 transfers an electron from one cytochrome complex to the next. However, if CoQ10 contacts oxygen before it interacts with the next cytochrome, it will give up the electron to oxygen and form super oxide. Under normal circumstances, this reaction will occur about 1-3% of the time. This small amount of oxidation is easily deactivated by common intra-cellular antioxidants. However, when there is an excess of glucose and/or FFA oxidation, an excessive amount of H^+ is pumped into the mitochondria by the electron transport chain (ETC) and this high concentration of H^+ inhibits the cytochrome complex from accepting the electron from CoQ10. CoQ10 then diverts these electrons to oxygen and generates excessive amounts of SO (super oxide). The kinases that are activated by SO then activate nuclear factors (proteins that control various genes: NF-kB, ATF, Fos/Jun) which activate the genes that control inflammation and apoptosis. These ROS, kinases, and gene products (cytokines, chemokines, adhesion molecules, etc) produce the ischemia, cellular proliferation, matrix accumulation and dysfunction, cellular necrosis, and apoptosis that lead to diabetic complications. In addition, the ROS, kinases, nuclear factors, and cytokines cross-stimulate each other, which accelerate this destructive process. Super oxide also inhibits the synthesis of nitric oxide (NO) and increases its destruction leading to a deficiency of this important compound. Nitric oxide is the primary, endogenous inhibitor of NF-kB (the inflammatory nuclear factor) and platelet aggregation; therefore, its deficiency allows this inflammation to proceed unchecked. In addition, nitric oxide is a very important vasodilator and its deficiency contributes to vasoconstriction and ischemia.

When diabetes does develop due to beta-cell destruction (Type 1 & 2 DM) or dysfunction (Type 2 DM), the hyperglycemia itself contributes to this oxidation and inflammation. Excess glucose will generate additional super oxide via auto-oxidation. It is also metabolized to sorbitol and advanced glycosylation end (AGE) products, both of which are activators of Protein Kinase C (discussed below). And, finally, it will induce a deficiency of myo-inositol. These processes will further aggravate the ischemic-inflammatory process. The high free fatty acids seen in the Metabolic Syndrome will also induce excessive VLDL secretion from the liver which will then have adverse effects on other lipoproteins and further accelerate macrovascular disease (atherosclerosis). This oxidation and inflammation is very detrimental to the beta-cells and is an important factor in their ongoing demise and, thus, worsening glucose control. There are specific fatty acids that seem to have particularly adverse effect on beta-cells. Ceramide, derived from sphingomyelin, is a direct inducer of nitric oxide. Nitric oxide induces apoptosis. Apoptosis in the atheromatous plaque is probably good because it leads to plaque shrinkage. However, apoptosis in the islets is bad because it destroys the beta-cells at the very time that they are most needed to overcome the insulin resistance in these patients. In addition, arachidonate is a substrate for the pro-inflammatory prostaglandins that also contribute to the destruction of the islets.

Oxidation and ROS:

Excessive oxidation of free fatty acids (beta-oxidation) and glucose (glycolysis → Krebs Cycle) generates NAD(P)H from $NAD(P)^+$, increasing the NAD(P)H to $NAD(P)^+$ ratio. This change in the redox state appears to have several detrimental effects. First, it inhibits further glucose and fatty acid oxidation. The partially degraded fatty acids remain bound to carnitine (the transporter that moves fatty acids from the cytoplasm into the mitochondria) thus inhibiting the transfer of more fatty acids into mitochondria. This inhibition of fatty acid degradation allows these toxic organic acids to accumulate in the cytoplasm. Carnitine may also be important in binding and neutralizing other organic acids and has been shown to prevent cellular damage in other toxic situations such as ischemia. Second, the increase in glucose and fructose oxidation leads to an increase in the production glyceraldehyde-3-P (GAP) (the 3-carbon intermediate in the middle of the glycolytic pathway), which is blocked from further metabolism by the altered redox state. Thus, the intracellular concentration of GAP is increased. This substance is known to be a major intracellular glycosylator of proteins (discussed below). In addition, GAP is a precursor for glycerol-3-P and subsequently diacylglycerol (DAG), which is a major activator of Protein Kinase C. Third, the equilibrium between pyruvate and lactate is shifted to lactate and, thus, increases the risk of acidosis. Any one of these metabolic alterations can lead to cellular dysfunction and eventual death. However, their combined effects can be even more devastating, especially when linked with the accumulation of sorbitol and ROS.

When NAD(P)H is metabolized via the mitochondrial electron transport chain, there is usually a small amount of the free radical super oxide (SO) produced (1-3%) (discussed above). However, when this pathway is overwhelmed by excessive oxidation of glucose and FFA's, the production of super oxide is greatly accelerated. Super oxide is also produced by (1) enzymes such as vascular NAD(P)H Oxidase, found on the surface of vascular cells, which is stimulated by oxidized LDL, TNFa and angiotensin II; (2) auto-oxidation of glucose itself; and (3) neutrophils and macrophages (via a NAD(P)H Oxidase related to the vascular oxidase) when exposed to glucose and FFA's. **Again**, ***super oxide*** **is a potent activator of Protein Kinase C.**

Sorbitol Pathway:

When glucose enters a cell, it is normally stored as glycogen or oxidized to carbon dioxide and water. However, if too much glucose enters the cell (i.e. cells in which insulin does not regulate glucose uptake), these usual pathways become overwhelmed and other pathways are activated. Certain cells contain an enzyme called "aldose reductase". In these cells, glucose is converted to sorbitol (a six carbon, non-ringed molecule with an "-OH" group on each carbon) and then to fructose. The fructose can then leave the cell and/or be metabolized in a variety of ways. Unfortunately, fructose is a more potent glycosylating agent than is glucose (discussed below), so that its accumulation can be very detrimental. Of course, the conversion to fructose will be inhibited by the high concentration of NADH typically seen in diabetes and this inhibition will increase the concentration of sorbitol even more. Therefore, sorbitol will accumulate in the cytoplasm and, because it cannot diffuse across membranes, this accumulation will lead to osmotic swelling of cells. This is particularly obvious in the lens and appears to be a significant factor in the blurring of vision that occurs in many diabetics after significant reductions or increases in their blood sugar level. However, this hyper-osmolarity also activates AMP-Kinase which activates DAG synthase which produces DAG (diacylglycerol) which stimulates Protein Kinase C which triggers the ischemic-inflammatory process discussed previously as well as the kinases JNK and MAPK which induce apoptosis. Apoptosis of nerves or beta-cells is obviously very detrimental.

Unfortunately, sorbitol is structurally very similar to myo-inositol, which is a 6-carbon, ringed molecule with an "-OH" group on each carbon. Myo-inositol is involved in a very important second-messenger pathway found in essentially all cells - the phosphatidyl-inositol (PI) pathway. PI is a minor, but very important, phospholipid found on the inner layer of plasma membranes. Even though PI is a minor component of plasma membranes, its turnover is very fast. Therefore, it requires an abundant supply of intracellular myo-inositol at all times to maintain normal function. In order to maintain this supply, myo-inositol is concentrated inside the cell by an active transport system. Unfortunately, sorbitol (and probably glucose) blocks this transport system. Thus, whenever sorbitol (or glucose) concentrations rise, myo-inositol levels fall, effectively starving the PI pathway of the substrate it needs to regenerate itself. Neurons may be particularly susceptible to this deficiency.

Normally, the PI pathway functions as follows:

1) Two additional phosphate molecules are enzymatically linked to the inositol in phosphatidylinositol (PI) making it: "PI 4,5-Bisphosphate" (PIP-2). Remember, one phosphate was already attached to inositol as the bridge connecting it to glycerol so that there are actually three phosphates attached to inositol.

2) A hormone binds to its receptor (at least 20 hormones have been identified that can stimulate this pathway) and activates Phospholipase-C with a “G-protein" acting as an intermediary.

3) Phospholipase-C cleaves Inositol Triphosphate (IP-3) from Diacylglycerol (DAG). Both IP-3 and DAG (discussed earlier) act as second messengers to activate a variety of enzymes and ion channels.

4) IP-3 is water-soluble and, therefore, moves into the cytoplasm of the cell where it binds to specific receptors on calcium-containing vesicles and stimulates the release of calcium into the cytoplasm. IP-3 is rapidly inactivated by the removal of its phosphates so that more IP-3 must be continually produced in order to continue the calcium release. Calcium modulates a number of cellular activities including activating Na/K-ATPase and plasma membrane calcium channels.

5) DAG remains bound to the plasma membrane where it activates Protein Kinase-C. Protein kinase C phosphorylates a number of enzymes, which contribute to ischemia and inflammation. One of these enzymes is also Na/K-ATPase.

6) DAG can undergo further metabolism by having arachidonic acid cleaved off which can then be used to produce prostaglandins. One of these prostaglandins (PGE_1) appears to directly activate Na/K-ATPase and is also pro-inflammatory.

7) Na/K-ATPase is vital to the maintenance of appropriate sodium and potassium concentrations inside the cell and of normal membrane potentials, which, in turn, are important for normal function of the Na/Ca pump and nerve impulse propagation.

It is apparent that this pathway is involved in many vital cellular functions and, if it is disrupted, there are likely to be dire consequences. It appears from several studies that in order for Na/K-ATPase to function normally under physiological conditions, both arms of this pathway (IP-3 and DAG) are required. It has been demonstrated that Na/K-ATPase activity is reduced in a number of diabetic tissues, particularly in the nerves where it is vital for nerve conduction. It is also known that sorbitol levels are high and myo-inositol levels are low in many of these same tissues. In animal studies, myo-inositol supplementation improves nerve conduction.

Non-enzymatic Glycosylation:

Whenever glucose comes in contact with protein or DNA molecules, it can become covalently linked via the aldehyde group on glucose and the terminal amino groups on arginine, lysine, and nucleic acids. The initial step in these reactions is the formation of a Schiff base, which is very labile, easily reversible, and reaches equilibrium within hours. Next, a slow rearrangement occurs to produce an Amadori product, which is much less labile. Reversal of this reaction is very slow and equilibrium occurs in about four weeks. This is the product that is commonly measured on hemoglobin (hemoglobin A1c) and albumin (fructosamine), which are used to evaluate patients' blood glucose control over the previous four to eight weeks.

The degree of glycosylation is determined by the concentration of glucose and the period of time that the protein or DNA is exposed to glucose (simple mass action). If these glucose molecules happen to block an important binding or enzymatic site, then they can disrupt normal function. In addition, glycosylation can create novel binding sites so that the protein binds to things that it normally would not (such as matrix proteins or antibodies).

Finally, glycosylated proteins can undergo an extensive series of oxidation-dehydration reactions and rearrangements to form complex advanced glycosylation end products (AGE-

products). The most important result of this reaction appears to be the formation of cross-links between proteins and/or strands of DNA via these AGE-products. These AGE cross-links clearly disrupt the normal function of these molecules. Since these reactions are irreversible, these AGE-products continuously accumulate for the life of the protein molecule, which is particularly harmful to proteins that are slowly metabolized (such as those found in the extracellular matrix). These cross-links frequently prevent the normal degradation and repair of these proteins.

An important interaction between glycosylation and the sorbitol pathway is that glyceraldehyde-3-P (GAP), other glycolytic intermediates, and fructose (all of which are increased by the sorbitol pathway) are potent glycosylators. In fact, fructose forms AGE-products about 10 times faster than glucose and fructosylation has been demonstrated in the lens proteins of diabetic humans.

Macrophages and endothelial cells express a receptor that recognizes proteins that have undergone this "AGE" process (RAGE – receptor for AGE). When these cells bind an AGE-protein, they activate Protein Kinase C, which leads to ischemia and inflammation.

Extracellular Matrix:

Extracellular matrix is composed of proteins (primarily collagen, elastin, and fibronectin) and proteoglycans (heparan sulfate being the most important in this context). Collagen and elastin are primarily structural proteins (like the steel beams of a building) while fibronectin is an adhesive protein (important in cell adhesion). Collagen synthesis is stimulated by TGF-beta (elevated in diabetes by Protein Kinase C) and inhibited by Nitric Oxide (reduced in diabetes). Proteoglycans, on the other hand, act as the fillers (like the bricks, plaster board, etc.) and traffic controllers (like the doors and windows) but their synthesis is inhibited by Protein Kinase C. Heparan sulfate is the most important proteoglycan in that it determines the size and charge-sieving property of basement membranes. It is also very important in inhibiting the uncontrolled replication of cells attached to the basement membrane. Heparan sulfate is held in place by binding to collagen but this binding is inhibited by glycosylation. Basement membranes in diabetic patients are characterized by an increase in collagen (due to both increased production and decreased degradation) and a reduction in heparan sulfate (due to reduced production and reduced binding to collagen). Therefore, the diabetic basement membrane is like a building with a lot of steel beams connected haphazardly but with no bricks, sheetrock, doors, or windows attached. Clearly, there is a lot of material present but it is incapable of fulfilling any worthwhile function. This is the hallmark of all diabetic tissues: a frequently massive increase in extracellular material that is unable to provide a physiological barrier. Even worse, eventually the accumulation of this useless material crowds out the functional components of the organ (the glomerulus in the kidney is a good example of this crowding). As discussed previously, many alterations in extracellular matrix can be attributed to the increase in sorbitol production (and its subsequent metabolism), glycosylation of the various proteins and DNA involved in synthesis and degradation of this matrix, and increased pro-inflammatory cytokines.

Specific Organ Systems

Nephropathy:

The kidneys undergo many of the same changes as the other organs but also present some unique features. First, they are the filtering system of the body and, because diabetes is a disease of the body's filters (basement membranes), the kidneys are particularly affected. Glomerular filtration is determined by three factors:

1) Perfusion pressure within the glomerulus
2) Glomerular capillary surface area
3) Integrity of the glomerular basement membrane

The glomerular perfusion pressure is controlled by the degree of constriction of the afferent and efferent arterioles of each glomerulus. Within days of developing diabetes, the control of these arterioles becomes dysfunctional and the perfusion pressure increases (probably due to both dilatation of the afferent and constriction of the efferent arterioles). The exact mechanism for these changes is complex and still controversial. The glomerular capillaries then dilate (increasing their surface area) and the kidneys enlarge, frequently increasing their size by 50%. The glomerular filtration rate (GFR) is similarly increased. This situation may continue for many years without any evidence of abnormal filtering selectivity (proteinuria) and can be rapidly reversed by tight blood glucose control.

If nephropathy progresses, proteinuria will develop. At first, it will be intermittent and in low concentration (micro-albuminuria, 20-300 mg/day) but later it becomes continuous, frequently reaching "nephrotic" levels (>3 grams/day) of protein excretion. Tight blood glucose control will frequently arrest the progression of nephropathy at the micro-albuminuric stage but will have variable effects during later stages. Proteinuria (specifically albumin) is a sign of the loss of basement membrane selectivity (both size and charge). Glomerular capillaries are fenestrated (porous) so that the only filtration barrier in the pores is the basement membrane. The severity of the proteinuria correlates with the increase in basement membrane material (collagen) within the glomerulus and the reduction in heparan sulfate. The cells that produce this basement membrane are the endothelial, epithelial, and mesangial cells (first cousins to the retinal pericytes). Eventually, this increase in matrix material and proliferation of the mesangial cells will crowd out the glomerular cells and capillaries resulting in a progressive fall in GFR. In addition, the large amount of filtered protein is toxic to the renal tubular cells and these also begin to die.

As nephrons are lost, patients become hypertensive if they are not already hypertensive. Controlling this hypertension is vital to slowing the progression of nephropathy. Controlling the blood glucose at this point does not seem to have a major impact on the progression of the disease but dietary protein restriction may be beneficial. Usually, the rate of loss of GFR is constant in an individual patient so that if the creatinine clearance is determined several times over a period of months, it can be used to predict when that patient will reach a particular level of renal function. To date, the only intervention that has been shown to change the rate of progression at this late stage is controlling the blood pressure. However, ACE-inhibitors (angiotensin converting enzyme) and ARB's (angiotensin receptor blockers) hold a unique place

in the treatment of diabetic nephropathy with gross proteinuria (>300 mg/day) because they have been shown to reduce the hyperfiltration, proteinuria, and the progressive loss of GFR almost independent of their antihypertensive effect. They are known to dilate efferent arterioles, which could explain some of their effects on hyperfiltration and proteinuria, but they also reduce the basement membrane abnormalities and inhibit cellular proliferation.

As the kidneys fail, the patients become anemic because of the loss of erythropoietin. This reduces the oxygen carrying capacity of the blood and worsens the ischemia of poorly perfused organs such as the retina. This anemia, along with the metabolic abnormalities associated with uremia, probably contributes to the rapid progression of retinopathy and neuropathy during this phase of the disease. In addition, activation of vitamin D (1-hydroxylase) is lost and phosphate excretion is reduced (inducing hyperphosphatemia). The reduction in intestinal calcium absorption and increased soft-tissue precipitation of calcium phosphate will induce secondary hyperparathyroidism. PTH is a pro-inflammatory hormone and also induces cardiac dysfunction contributing to the atherosclerosis and cardiomyopathy typical of diabetes and renal failure.

Retinopathy:

Retinopathy eventually affects over 80% of diabetic patients and is the leading cause of blindness in this country. The incidence and severity of the retinopathy is related to the duration of diabetes and the level of blood glucose control. Retinopathy follows a fairly consistent sequence of events, which ultimately can lead to either one or both of the typical clinical presentations: **macular edema and/or neo-vascularization**. One of the earliest signs of hyperglycemia-induced vascular dysfunction is the accumulation of fluorescein (a dye used by ophthalmologists to examine the eyes) in the retina and vitreous. The accumulation in the vitreous is due primarily to dysfunctional pigment epithelial cells (which are known to accumulate sorbitol), whose job it is to remove a variety of toxic substances from the vitreous. Later, there is leakage of fluorescein and plasma proteins from the retinal capillaries coincident with thickening of the basement membrane, loss of heparan sulfate, and apparent endothelial cell dysfunction.

Pericytes are aldose reductase-containing cells that are attached to the outer surface of capillaries. They produce basement membrane and inhibit endothelial cell replication and migration. There is a high concentration of pericytes on normal retinal capillaries. The earliest morphological changes seen in diabetic eyes are an increase in basement membrane thickness and a reduction in the number of pericytes. The oxidative stress seen in diabetes will also lead to vasoconstriction and inflammation in these capillaries. Eventually, the pericytes die and the capillaries become totally denuded of these cells. The endothelial cells are then allowed to replicate and migrate into clumps of cells called microaneurysms (these are not true aneurysms since they do not necessarily have a lumen). The loss of heparan sulfate (which normally inhibits cell migration and proliferation) from the basement membrane of these capillaries also contributes to this increase in endothelial cell proliferation. Exudates and intra-retinal hemorrhages, visible with an ophthalmoscope, are the result of leakage of plasma proteins and blood from these defective capillaries. If this leakage of material occurs between the retina and choroid layers of the eye, the retina will lose its nourishment and die. If this swelling occurs near the macula, it is called **macular edema** which is the leading cause of vision loss in diabetic patients.

Ultimately, avascular capillaries (ghosts) can be identified which no longer contain blood. These are just the remains of the basement membrane without pericytes or endothelial cells. Why the endothelial cells are lost is not clear. Replication of endothelial cells is not inhibited by hyperglycemia, in fact, they appear to replicate abnormally fast as noted above. There is currently no firm evidence of cell toxicity, which could explain the endothelial cell death, but the diabetic environment is clearly potentially lethal. Aldose reductase has been demonstrated in other endothelial cells but not in retinal endothelium as yet. It is clear that the endothelial cells' ability to produce anti-coagulant and anti-platelet factors is reduced by hyperglycemia and this may allow these capillaries to become obstructed and thus lead to the demise of the endothelial cells. Oxidative stress is also a strong possibility. Another possibility is that the loss of pericytes and heparan sulfate allow the endothelial cells to replicate and migrate into clumps (microaneurysms), which occlude the capillaries.

The end result of these events is ischemia of various areas of the retina. Angiogenic factors (VEGF, etc) are then released that stimulate new blood vessel formation (neo-vascularization). All new vessels are more leaky than old vessels but new diabetic vessels are even more leaky than new non-diabetic vessels. This makes hemorrhage very likely. Moreover, these vessels frequently grow out into the vitreous rather than stay within the retinal layers. Thus, when they bleed, the blood is released into the vitreous and the patient can no longer see (depending on the severity of the bleed). Blood in the vitreous can also obstruct the drainage of vitreous fluid and cause severe, acute glaucoma and retinal death. As inflammatory cells clear the blood cell debris, they lay down scar tissue within the vitreous. This scar tissue contracts and will frequently pull the retina away from the choroid layer of the eye from which the retina receives its nutrients. If the retina is not surgically reattached to the choroid very quickly, it will die. Even if VEGF does not induce substantial neo-vascularization, it will increase vascular permeability and aggravate macular edema, thus increasing the risk of vision loss.

One of the most effective treatments to prevent the complications of macular edema and neo-vascularization (besides early control of the blood glucose and prevention of the whole process) is laser photocoagulation therapy. Laser is used for three purposes. First, it is used to destroy the new vessels or edematous areas in order to prevent further leakage or bleeding. Second, laser is used to burn and destroy up to 80% to 90% of the peripheral retina (while preserving central vision) in order to reduce the number of cells requiring blood. This reduces the degree of ischemia and the synthesis of VEGF by these cells and, thus, reduces vascular permeability and prevents new vessel formation. Finally, laser therapy welds the retina to the choroid layer and makes detachment of the retina almost impossible.

Neuropathy:

Many diabetic patients will eventually develop some degree of neuropathy (identified by either symptoms or abnormal nerve conduction studies). The incidence and severity of neuropathy will increase with the duration of diabetes and level of blood glucose. Animal and human studies demonstrate detectable alterations in nerve function within days of developing diabetes, much of which is reversible with good blood glucose control. This is not surprising because of the tremendous importance of Na/K-ATPase activity in nerve conduction, which, in

turn, depends on a well-functioning phosphatidyl-inositol system. However, with continuing hyperglycemia, all components of the nerve (axons, Schwann cells, perineural, and endoneural cells) become involved since they all contain aldose reductase and have insulin-independent glucose transport. There is eventual atrophy of nerve fibers, demyelination, infarction of individual nerves, and breakdown of the blood-nerve barrier. Long nerves are the most susceptible to these events so peripheral neuropathy involving the feet is frequently the first clinical sign. Eventually all nerves can be affected so that almost any conceivable neurological symptom relating to the peripheral or autonomic nervous systems can be seen in diabetes.

The most catastrophic event associated with peripheral neuropathy is probably the loss of a foot or leg. This is almost always preventable if the patient has adequate macro-circulation to the limb (i.e. minimal atherosclerosis). The typical scenario is that the patient will damage the foot (wrong shoes, pebble in the shoe, nail through the shoe) and ignore it until it becomes a large, open wound full of pus. Since the patients cannot feel their feet, they don't worry about them. You must remember, however, that even at this stage, most infections can be treated with drainage and antibiotics if there is adequate circulation. The fundamental pathophysiology of a neuropathic ulcer is:

pressure --> ischemia --> inflammation!!

These patients usually have adequate macro-circulation but they put continuous mild pressure on a specific part of their foot (like the heel or ball of the foot) which prevents adequate capillary flow (the area blanches). After several hours, the dermal layer of the skin becomes ischemic and then inflamed (red and warm). Normally, this ischemia would cause excruciating pain and the individual would remove the pressure from this area. However, the diabetic patient will not feel the pain and will continue to apply the pressure. If this pressure continues or is applied intermittently for extended periods of time, then the epidermal layer becomes detached from the inflamed dermis and is sloughed off, thus forming an ulcer. The obvious treatment for this pathological process is **to get the patient off of their feet entirely until the lesion completely heals!!** Therefore, it is very important to TEACH PATIENTS TO LOOK AT THEIR FEET so that problems can be treated early. Some offices are currently using "heat sensing" devices in order to detect early signs of inflammation. This allows appropriate therapy to be instituted before an ulcer actually develops.

Autonomic neuropathy is probably the most debilitating problem for diabetic patients in their day to day living. They can develop orthostatic hypotension (some patients cannot stand without fainting), impotence, and gastro-intestinal motility dysfunction leading to nausea, vomiting, diarrhea, constipation, and/or severe abdominal pain. The treatment of all of these symptoms is extremely difficult so prevention is of the utmost importance. These symptoms frequently develop in relatively young individuals and can keep them bedridden for years.

Finally, diabetic patients can develop mono-neuropathies involving any nerve in their body. Mini-infarcts of the nerve or nerve compression of some type typically produce these mono-neuropathies. Nerve entrapment (for example - carpal tunnel syndrome) is made more likely by the excess of matrix material commonly seen in diabetes. These neuropathies can produce pain, numbness, paresthesias, and focal muscle weakness. Besides being troublesome in themselves, they can be confused with other diseases such as a stroke, heart attack, cholecystitis, or any other

pathology that produces pain. This confusion can lead to unnecessary studies and even surgery.

Macrovascular disease:

Atherosclerosis of the large arteries is a common complication of both types of diabetes. As noted earlier, it probably accounts for the majority of deaths in diabetic patients. This is especially true today because we are able to treat the triopathy better and are preventing most renal deaths. Now these individuals are dying from large vessel disease.

Diabetics are subject to the same risk factors as non-diabetics (dyslipidemia, hypertension, family history of coronary disease, smoking, etc.) but Type 2 DM is almost always (>90%) associated with insulin resistance, which is usually associated with hypertension and a specific dyslipidemia (high triglycerides and low HDL). These are two very strong risk factors for atherosclerosis that are not commonly seen in Type 1 DM prior to the development of nephropathy. However, these risk factors do not fully explain the increased risk of atherosclerosis in diabetes. It has been proposed that there are at least three factors specific to diabetes that contributes to this increased risk:

1) The leaky endothelium permits higher concentration of lipoproteins into the sub-intimal layer of the artery.
2) Glycosylation of lipoproteins inhibits their normal metabolism and increases their abnormal metabolism (as discussed below).
3) Hyperglycemia (probably through glycosylation, oxidation, and abnormalities in inositol metabolism) produces a number of abnormalities in lipoproteins, platelets, fibrinolysis (increases PAI-1), and endothelial function.

An interesting relationship between basement membrane abnormalities and atherosclerosis in non-diabetics has been discovered. Non-diabetics who have microalbuminuria (presumably due to a genetic alteration in their basement membrane composition) have an increased risk of developing atherosclerosis. This finding points to the importance of maintaining a normal endothelial barrier as well as the close connection between the glomerular basement membrane and the arterial basement membrane.

Diabetic patients are recognized to have a high "oxidant-load"; that is, they generate excessive amounts of free radicals. This is probably because of the high levels of NADH, NADPH, and metabolic intermediates in diabetic cells as discussed earlier. There are also a number of cellular oxidases that are activated in diabetics. This "oxidant-load" is especially high in diabetic smokers. The final irreversible step of protein glycosylation and cross-linking is, in fact, a free radical/oxidation reaction. LDL has been shown to be glycosylated in diabetic patients which subsequently increases its binding to extracellular matrix in arterial walls (via crosslinks) as well as increases its uptake into foam cells via the scavenger receptor, while reducing its normal clearance by the liver. Even in non-diabetics, it is now apparent that oxidation of LDL without glycosylation is vital to its being taken up by foam cells to form plaques. Drugs are being developed to block the cross-linking of "AGE products" but, in the meantime, anti-oxidants (vitamin E: 400-1000 U/day; vitamin C: 250-1000 mg/day; selenium 200 ug/day; alpha-lipoic acid 400 to 1200 mg/day; borage oil 3,000 mg daily; vitamins B1, B6, B12; folic acid) may have a role to play in the prevention of several diabetic complications

including atherosclerosis.

Blood Glucose Control and Chronic Complications:

There are a number of randomized, controlled trials, including the DCCT and UKPDS studies, which demonstrate that improving blood glucose control reduces the incidence of neuropathy, retinopathy, and nephropathy. Improving blood glucose control also slowed the progression of the triopathy in those patients who already had mild to moderate complications. These data support observational studies that have shown that patients with lower HgbA1c values (better glucose control) have reduced cardiac events and mortality.