

Mitochondrial Factors in the Pathogenesis of Diabetes: A Hypothesis for Treatment

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Abstract

A growing body of evidence has demonstrated a link between various disturbances in mitochondrial functioning and type 2 diabetes. This review focuses on a range of mitochondrial factors important in the pathogenesis of this disease. The mitochondrion is an integral part of the insulin system found in the islet cells of the pancreas. Because of the systemic complexity of mitochondrial functioning in terms of tissue and energetic thresholds, details of structure and function are reviewed. The expression of type 2 diabetes can be ascribed to a number of qualitative or quantitative changes in the mitochondria. Qualitative changes refer to genetic disturbances in mitochondrial DNA (mtDNA). Heteroplasmic as well as homoplasmic mutations of mtDNA can lead to the development of a number of genetic disorders that express the phenotype of type 2 diabetes. Quantitative decreases in mtDNA copy number have also been linked to the pathogenesis of diabetes. The study of the relationship of mtDNA to type 2 diabetes has revealed the influence of the mitochondria on nuclear-encoded glucose transporters and the influence of nuclear encoded uncoupling proteins on the mitochondria. This basic research into the pathogenesis of diabetes has led to the awareness of natural therapeutics (such as coenzyme Q10) that increase mitochondrial functioning and avoidance of trans-fatty acids that decrease mitochondrial functioning.

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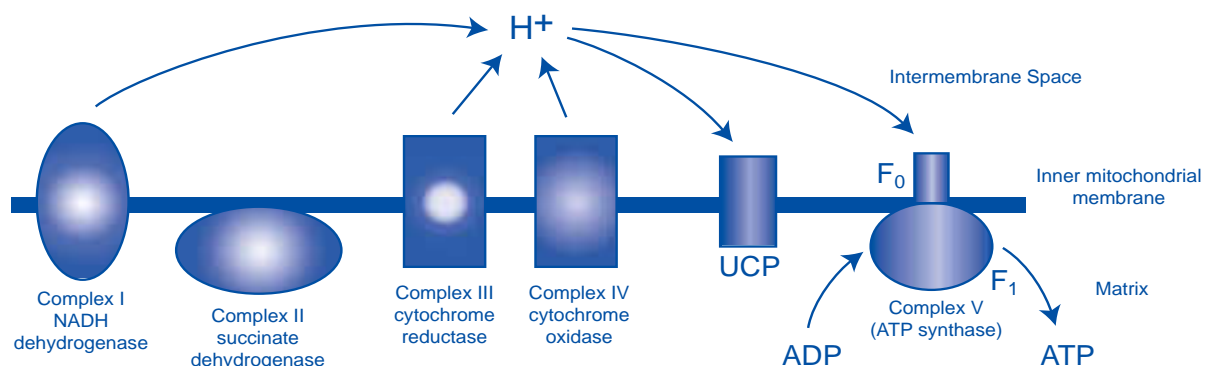
Introduction

In reviewing the effect of high-dose chromium supplementation on blood sugar regulation and type 2 diabetes,¹ it becomes apparent that various mitochondrial factors are of paramount importance in the pathogenesis of diabetes. Type 2 diabetes is characterized as a heterogeneous disease, which affects over 3 per 1,000 persons in the United States and accounts for over 80 percent of the diabetic patients seen by physicians. The cause of this disease has been ascribed to obesity as well as genetic factors. It is considered a heterogeneous disease due to the multiplicity of factors that cause the observed phenotype. For many years researchers have argued that type 2 diabetes is a problem of insulin sensitivity, focusing on the insulin receptors in the peripheral tissue. Other researchers have focused on the dysfunction of the beta-cells, similar to type 1 diabetes, as the primary dysfunction in type 2. Today it is recognized that both factors contribute to the disease. Beta-cell malfunction can be traced to various levels of qualitative and quantitative mitochondrial dysfunction. Mitochondrially mediated type 2 diabetes is often genetically expressed via mutations in the mitochondrial DNA (mtDNA). A second aspect of mitochondrial contribution involves the quantitative decrease in mtDNA in response to oxidative stress. There is evidence of a more global effect of mitochondrial dysfunction at the glucose transporter level.

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Figure 1. Mitochondrial Respiratory Chain



Mitochondrial Structure and Function

Mitochondria are semi-autonomous organelles found in every cell in the human body. A single cell can contain from 200 to 2,000 mitochondria.^{2,3} The number of mitochondria in specific cell types varies considerably, although within a given cell type the number is closely regulated.⁴ The mitochondria, often referred to as “the power house of the cell,” are the source of at least 90 percent of the energy generated in the cell.⁵ The majority of this energy (80%) is produced as adenosine triphosphate (ATP) by oxidative phosphorylation (OXPHOS). A series of enzyme catalyzed redox reactions leads to the production of ATP along with reactive oxygen species (ROS). ATP is produced by a series of five multi-subunit enzymes or complexes (Figure 1). Complex I (ubiquinone NADH dehydrogenase) is one of four transmembrane multienzyme complexes within the inner mitochondrial membrane (complexes I, III, IV, V). Complex I is responsible for the oxidation of NADH, pumping four protons (H^+) into the intermembrane space while reducing ubiquinone. Complex II (succinate dehydrogenase) oxidizes metabolites such as succinate into malate, liberating reducing equivalents (electrons)

that are shuttled to complex III via ubiquinone. Complex II is unique in that it is not a transmembrane protein and has no mitochondrial genetic coding. Complex III (ubiquinol-cytochrome-c reductase) receives electrons shuttled by ubiquinone, liberating two protons (H^+) in the process. Complex IV (cytochrome-c oxidase) is a transmembrane complex that receives electrons, reducing oxygen from water, and producing two protons H^+ in the process. As protons are pumped out of the matrix, each complex moves electrons along the chain. The ultimate phosphorylation of ADP to ATP occurs because of a proton gradient created by the oxidation of various compounds by the first four complexes. The proton gradient creates a transmembrane potential used by complex V (ATP synthase, F_1F_0 ATPase) to drive the synthesis of ATP via the three-step rotation F_1 around F_0 , causing conformational changes in F_1 that activate catalytic domains, producing ATP.

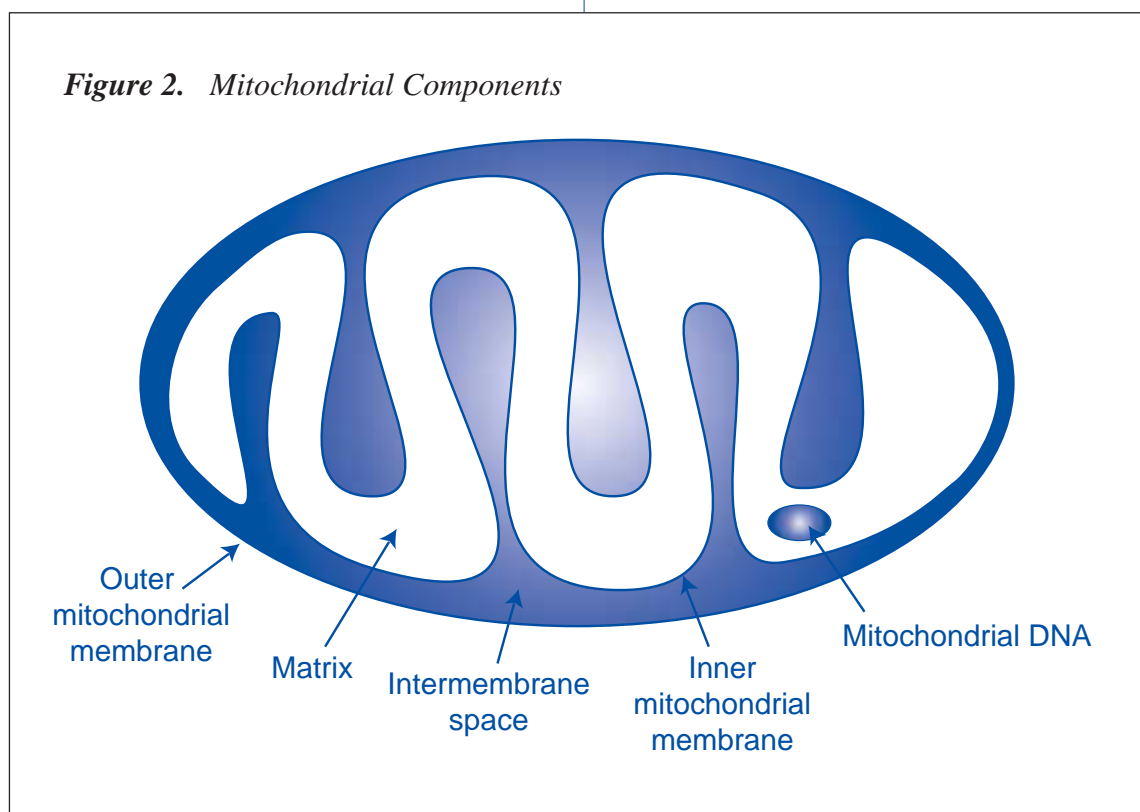
The majority of mitochondrial proteins are synthesized in the nucleus and shuttled to the mitochondria. These organelles are referred to as semi-autonomous because, unlike any other organelle, they have their own mtDNA that codes for the production of four of the five enzyme complexes critical for oxidative phosphorylation. The human mitochondrial genome is comparatively small, consisting of only 16,569 base pairs, about

10^5 less than in the cell nucleus. The mitochondrial genome encodes for 13 proteins involved with oxidative phosphorylation as well as 22 tRNAs and 2 rRNAs involved in synthesis of these mitochondrial complexes.

Each mitochondrion contains 2-10 copies of the circular, supercoiled, double-stranded DNA found unprotected within the inner mitochondrial membrane. This circular DNA is attached, at least transiently, to the inner mitochondrial membrane (Figure 2).⁶

DNA, such as protective histones, chromatin structure, and introns,¹⁰ and second, the proof reading apparatus for mtDNA is much less efficient than that of nDNA.^{11,12} Mutations in mtDNA can have a significant detrimental effect due to decreased noncoding regions. Mitochondrial DNA has two noncoding areas – a control region characterized by three hypervariable areas as well a displacement region (D-loop). The D-loop region controls replication of the mtDNA.

Figure 2. Mitochondrial Components



The close proximity of mtDNA to the harmful ROS by-products of oxidative phosphorylation makes mtDNA more vulnerable to attack. During oxidative phosphorylation, five percent of the oxygen used in respiration is converted to superoxide anions or other ROS.⁷ It is estimated mtDNA has a mutation rate 10-20 times higher than nuclear DNA.^{8,9} Two factors contribute to the vulnerability of mtDNA to mutation as compared to nuclear DNA (nDNA). First, coupled with the close proximity to ROS, mtDNA is also lacking the protective strategies associated with nuclear

Mitochondrial Function in Beta-cell Insulin Secretion

It has been recognized for a number of years that pre- and type 2 diabetic patients have an impaired ability of the pancreatic beta-cells to secrete insulin.^{13,14} The homeostasis of glucose and insulin is based on the ATP generated by glucose in the beta-cell. Glucose-stimulated insulin secretion (GSIS) has been characterized by its pulsatile nature as generated by oscillations in the ATP/

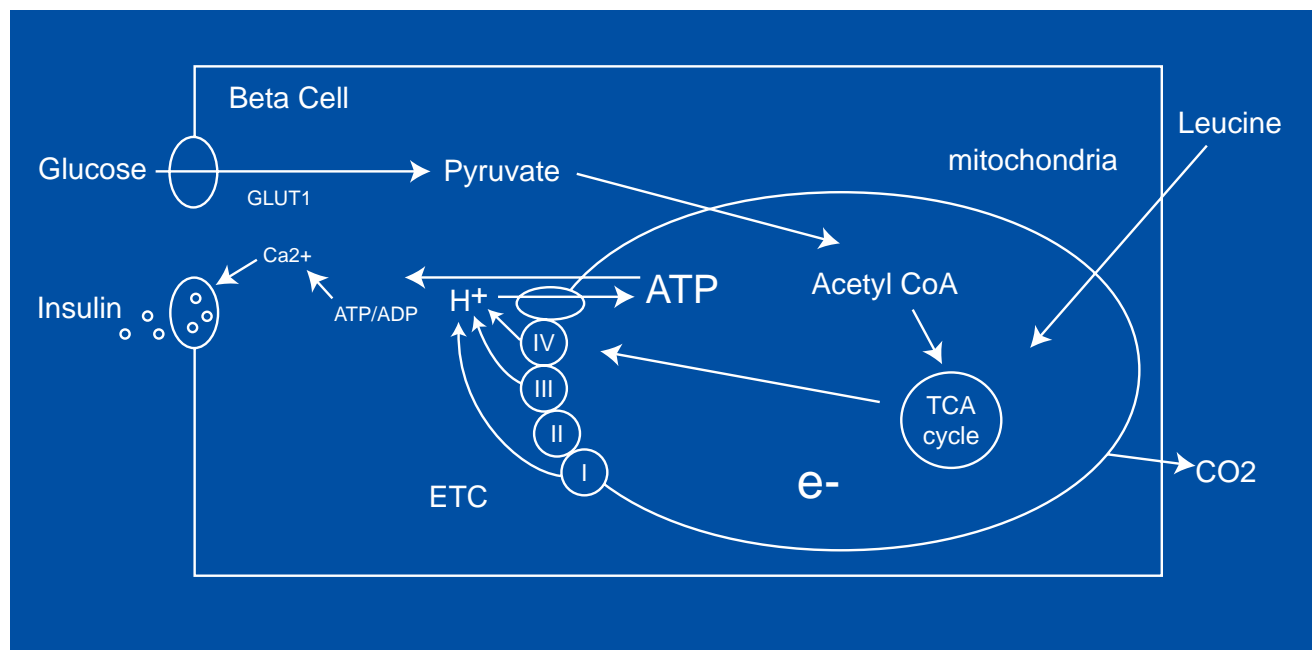
ADP ratio.¹⁵ A series of steps must be completed inside and outside the mitochondria before insulin can be secreted (Figure 3).

Glucose is transported across the cellular membrane by glucose transporters (GLUT), primarily GLUT-1, although some low affinity GLUT-2 transporters are expressed.¹⁶ Glycolysis transforms glucose to pyruvate, of which more than 90 percent is shuttled into the mitochondria.¹⁷ As pyruvate is further broken down by the tricarboxylic acid (TCA) cycle in the mitochondria, some ATP is liberated, as well as reducing equivalents such as FADH and NADH. The NADH and FADH shuttle electrons to the electron transport chain (ETC), which produces more ATP. The ratio of ATP/ADP increases as processing of the glucose increases. This increase in ATP/ADP ratio causes the ATP-sensitive K^+ channels to close, causing depolarization of voltage-sensitive Ca^{2+} channels.¹⁸⁻²⁰ The depolarization causes an influx of Ca^{2+} into the cytosol that triggers the exocytosis of insulin secretory vesicles produced by the Golgi complex.²¹ The processing of proinsulin to insulin within the secretory granules of the Golgi

complex is dependent on critical levels of pH (3.5-7.4) as well as ATP.²² Proinsulin, a precursor of insulin with little hormonal activity, is converted to insulin by the removal of the connecting C-peptide. Disruptions in processing lead to increased levels of proinsulin and decreased levels of insulin in prediabetic as well as type 2 diabetic patients.²³ Standard radioimmunoassay tests, which do not distinguish between inactive proinsulin and insulin levels, have been found to miscalculate insulin levels by almost 40 percent when compared with an immunoradiometric assay, which can distinguish insulin from proinsulin.²⁴ High proinsulin levels can predict type 2 diabetes more than three years in advance of disease.²⁵⁻³⁰

There are a number of other substances that can stimulate insulin release, such as leucine and long-chain acetyl-coenzyme A. Leucine, like glucose, stimulates insulin; but, unlike glucose, it is used directly by the mitochondria producing acetyl-coenzyme A for the TCA cycle.³¹ Long-chain acetyl-coenzyme A is also produced by glucose metabolism and has been shown to stimulate insulin secretion.¹⁵

Figure 3. Beta-cell Mitochondrial System



UCP in Type 2 Diabetes

Uncoupling proteins (UCP) drain protons from the intermembrane space directly into the mitochondrial matrix, bypassing ATP synthase (Figure 1). UCP are coded for via nuclear DNA and transported to the inner mitochondrial membrane. The members of this superfamily of mitochondrial anionic carrier proteins function as proton channels. This results in a decreased membrane potential, which is defined as the electrochemical potential difference of protons across the inner mitochondrial membrane.³² This means that in the case of UCP-1, increased induction leads to thermogenesis, while in the case of UCP-2 and -3, increased induction is thought to modulate ROS.³³

Uncoupling protein-2 is expressed in the pancreatic beta-cells. Its up-regulation is associated with decreased ATP production, closure of ATP-sensitive potassium channels, and impairment of glucose-stimulated insulin secretion. *In vitro* studies have demonstrated an inverse relationship between suppression of GSIS and UCP-2 expression.³⁴ Beta-cells subjected to experimentally-induced oxidative stress were found to combat H₂O₂ toxicity through the induction of UCP-2.³⁵ Zhang et al,³⁶ using UCP-2 deficient mice, discovered they had increased ATP production and GSIS. In contrast, diabetic ob/ob mice have been found to have an increase in UCP-2 expression. The increase in UCP-2 might be a consequence of an underlying inflammatory process, which has been implicated in the pathogenesis of type 2 diabetes. Studies have shown obese mice such as ob/ob and Zucker fa/fa have unregulated inflammatory transcription factor NF-kappaB. In obese mice, upstream inactivation of the activator of NF-kappaB, I-kappa B, has led to the reversal of hyperglycemia, hyperinsulinemia, and dyslipidemia.³⁷ Although increases in UCP-2 are associated with the sequelae of type 2 diabetes, it seems the expression of UCP-2 is a consequence of up-regulation of ROS via an underlying inflammatory condition. One practical aspect of the above is decreased ROS by increased antioxidant supply, which may result in the down-regulation of UCP, leading to increased ATP and a decrease in symptoms of type 2 diabetes.

Mitochondrial Genetic Factors of Type 2 Diabetes – Qualitative Changes in mtDNA

While research has largely focused on the analysis of insulin receptors and pancreatic beta-cell dysfunction, it has become apparent that mutations in mitochondrial DNA can also lead to type 2 diabetes. A mitochondrial disorder can result from the substitution, deletion, or duplication of mtDNA bases. It has been estimated that 0.1-9.0 percent of the diabetic population is affected due to a mutation in mtDNA.³⁸ There have been over 40 different mtDNA mutations catalogued that result in diabetes mellitus.³⁸ Many of these mutations are also associated with more serious mitochondrial disorders such as mitochondrial encephalopathy, lactic acidosis and stroke-like syndrome (MELAS), mitochondrial myopathy (MM), neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP), and Leber's hereditary optic neuropathy (LHON). Many of the mutations are associated with type 1 diabetes, yet a number have been found to result in type 2 diabetes. Maternally-inherited type 2 diabetes was one of the first mitochondrially inherited diabetic conditions to be identified.^{39,40} It is the most common form of mitochondrial type 2 diabetes accounting for 1.5 percent of diabetes cases and is caused by an adenine-to-guanine transversion mutation in the tRNA_{LEU} gene.⁴¹ Other mutations found to correspond to the type 2 diabetes phenotype are spread throughout the mitochondrial genome.⁴²

MtDNA Mutation: Heteroplasmic versus Homoplasmic

When damage to mtDNA is not repaired, it can result in a cascade of events ultimately leading to a number of diseases. A mitochondrial disorder can result from the substitution, deletion, and duplication of mitochondrial DNA bases and depletion of mtDNA copies. To add to the complexity of mitochondrial disorders, they can arise from one mtDNA mutation or from a number of independent mutations that in turn can lead to more than one disease type. The naturally occurring circle of mtDNA is also referred to as wild-type

mtDNA. The number of mutations can increase in a particular tissue, while not being reflected in other parts of the body. The mixtures of wild-type and mutant mtDNA coexisting in the same mitochondria are referred to as heteroplasmic mutations. Mitochondrial mutations can also be homoplasmic in nature when cellular mitochondria contain all of the same mutant mtDNA.

Repeated cell division leads to the separation of heteroplasmic and homoplasmic cell lines in a phenomenon of random segregation. Mutant mtDNA increases with aging and the cellular energy capacity can decrease. This decrease in turn affects the threshold of minimal cell function.³³

Mutation Threshold

Although cells may harbor mutant mtDNA, the expression of disease is dependent on the percent of mutations. Modeling confirms that an upper threshold level might exist for mutations beyond which the mitochondrial population collapses with a concomitant decrease in ATP.⁴³ This decrease in ATP results in the phenotypic expression of disease.⁴⁴⁻⁴⁶ It is estimated that in many patients with clinical manifestations of mitochondrial disorders the proportion of mutant DNA exceeds 50 percent.⁴⁶

The concept of a threshold level of mutation can be illustrated in a mutation of base pair 8993 in the ATPase gene. This heteroplasmic mutation can result in the phenotypic expression of diabetes, NARP, or Leigh Disease (named for its discoverer in 1951).⁴⁷ The phenotypic expression seems to be contingent on the level of mitochondrial mutation. If the mutation rate is below 60 percent it can express itself as diabetes. When the mutation rate is 60-90 percent the phenotypic expression results in the diagnosis of NARP. And, when expression of this mitochondrial mutation is over 90 percent the condition of Leigh Disease manifests in the patient,³⁸ a condition characterized by psychomotor retardation, dysphagia, hypotonia, ataxia, weakness, external ophthalmoplegia, convulsions, blindness, and deafness, with a usual prognosis of less than two years.

The mutation in base pair A3243G, coding for tRNA leucine 1, is associated with type 2 diabetes plus deafness.⁴⁸ Researchers have found a strong association between the level of mutational heteroplasmy and disease diagnosis.⁴⁹ Increased percentages of mutant mtDNA in muscle cells (up to 71%) can lead to mitochondrial myopathy.⁵⁰ Levels of heteroplasmy over 80 percent may lead to recurrent strokes,⁵¹ and mutation levels of 95 percent have been associated with MELAS.⁴⁶

Tissue Threshold-OXPHOS Capacity

Regardless of the type of mutation or the amount of heteroplasmy in affected mitochondria, unrepaired damage leads to a decrease in ATP, which in turn causes the phenotypic manifestation of disease. The manifestation of disease not only depends on the ATP level but also the tissue affected. Various tissues have differing levels of demand on OXPHOS capacity. For example, the tissue threshold for muscle or neuronal tissue is thought to be as high as or higher than 90 percent for damaged mtDNA.⁵² This means the proportions of damaged mtDNA in the total cellular pool must exceed 90 percent before onset of biochemical abnormalities appear. Another example illustrating the relative thresholds of various tissues was reported in the classic mitochondrial disorder of Leber's hereditary optic neuropathy, in which mitochondrial respiratory demand from highest to lowest was neurons, skeletal muscle, cardiac muscle, kidney, and liver, respectively.⁵³ Thus, the threshold of malfunction in a given tissue is dependent on the energy demand and the sensitivity of the tissue to mitochondrial dysfunction.

For biochemical malfunctions to occur, threshold values for complex I dysfunction have been calculated to be 70-80 percent for muscle, liver, and kidney, 64 percent for the heart, and 50 percent for the brain. For complex III dysfunction, threshold values were found to be high and showed little tissue variation. For complex IV dysfunction, threshold values for the heart and skeletal muscle were calculated to be 67 percent, while kidney and brain were 86 percent. The

sensitivity of threshold levels to demand can be illustrated with a complex IV example. An 80-percent decrease in activity of complex IV will lead to a small decrease in liver function, while the heart will experience a 40-percent decrease in function.⁵⁴

MtDNA Depletion and Glucose-stimulated Insulin Release – Quantitative Changes in mtDNA Copies

A number of studies have demonstrated the critical action of ATP in GSIS. Mitochondrial dysfunction as seen in the depletion of mtDNA might also be critical to the pathogenesis of type 2 diabetes. A growing body of evidence supports the supposition that the quantitative depletion of mtDNA, once thought to be a consequence of type 2 diabetes, could be a causative factor in pathogenesis. Early studies with the Goto-Kakizaki rat, a genetic animal model of type 2 diabetes with impaired insulin secretion, found that the mitochondria of beta-cells were decreased in volume, while the islet tissue had an increased number of mitochondria per unit area, but a decrease in mtDNA copies. The decrease in mtDNA (approximately 50 percent per mitochondrion) was not found to be associated with any major deletions or mutations. The decrease in mtDNA was observed in the adult rat tissue (four-month old) but not in fetal tissue.⁵⁵ These results suggest a connection between GSIS and the somatic progression of mtDNA depletion. This study and others show a correlation between mitochondrial function and type 2 diabetes. The data seem to suggest that metabolic dysfunction inside the mitochondria produces increased ROS, leading to decreased mtDNA, resulting in decreased insulin secretion.

The mitochondria are among the chief producers of oxygen free radicals within the cell.⁵ The electron transport chain constantly produces superoxide radical anions, which, in the case of mitochondrial dysfunction, cause the escape of electrons that readily form hydroxyl radicals and hydrogen peroxide from superoxide. Mitochondria can be affected by a number of substances that produce oxidative stress. Studies have shown the ingestion of the “synthetic heroin,” 1-methyl-

4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), leads to an increase in ROS and the inhibition of mitochondrial complex I through the metabolism of this protoxin to 1-methyl-4-phenyl-pyridinium ion (MPP⁺).^{56,57} Increased oxidative stress produced by ROS has been linked to mtDNA damage.⁵⁸

Another example of exogenously induced oxidative damage to mtDNA⁵⁹ can be illustrated with streptozotocin to create diabetes in test animals. Streptozotocin increases ROS causing damage to mtDNA and suppression of mitochondrial transcription.^{60,61} Hydroxyl radicals have been shown to attack nucleosides of DNA, producing oxidized products such as 8-hydroxy-2'-deoxyguanosine (8-OHdG). This ROS attack, although known to damage nuclear DNA, can attack mtDNA at rates 3-23 fold higher.^{62,63} Suzuki et al⁶⁴ found mutations in relation to levels of 8-OHdG in 22 patients with type 2 diabetes analyzed for mtDNA mutations. Levels of mtDNA mutations increased in proportion to the amount of 8-OHdG in mtDNA. Furthermore there was a significant correlation between the severity of diabetic complications and 8-OHdG content. The higher rate of 8-OHdG production corresponds to other reports of increased oxidative DNA damage associated with diabetic complications.⁶⁵⁻⁶⁷

A number of *in vitro* studies have demonstrated that critical mtDNA levels along with effective oxidative phosphorylation are required for the regulation of GSIS. *In vitro* studies employing specific inhibitors of the mitochondrial respiratory complexes inhibited glucose-stimulated insulin secretion. Mitochondrial inhibitors such as rotenone for complex I, antimycin A or sodium azide for complex III, and cyanide for complex IV, were all found to inhibit insulin release from pancreatic islet cells.^{68,69} Sodium azide was found to block insulin secretion despite high levels of Ca²⁺ in the cytosol.^{69,70} (MtDNA depletion has also been shown to inhibit the glucose-stimulated increase in the intracellular free Ca²⁺ content.⁷¹) The results obtained through the specific inhibition of various complexes, although appearing to demonstrate that OXPHOS is critical for GSIS, do not unequivocally prove a direct relationship. The inhibitory compounds used for study are cytotoxic

themselves, resulting in effects that are irreversible. Therefore, it is possible that the mechanisms involved may not be pertinent to normal cells.

The most elegant studies to analyze the connection between mtDNA depletion, OXPHOS dysfunction, and GSIS have produced mtDNA-deficient cells by depleting cellular mtDNA with low levels of ethidium bromide (EB) (0.1-2 ug/mL).⁷² EB, an inhibitor of the synthesis of DNA and RNA, intercalates into both nuclear and mitochondrial DNA, but due to less efficient repair mechanisms in the mitochondria, mtDNA is eliminated or rendered dysfunctional.⁷³⁻⁷⁵ These cells depleted of mitochondrial DNA (rho(0)) have been found to have no respiratory complexes and consequently no oxidative phosphorylation, since these cells rely on glycolysis for ATP production. At lower levels of ethidium bromide, cell lines can recover into cells with functional mitochondria and mtDNA.

The lack of a functional electron transport chain results in the inability to generate ROS, which has been found to be an integral part of a number of mitochondrial signaling pathways, such as glucose-stimulated insulin secretion.⁷⁶ As noted in the section on mitochondrial function, membrane depolarization is a critical factor in GSIS. The ability of the plasma membrane to depolarize remains functional in the rho(0) cells as evidenced by the release of insulin with the depolarizing agent KCl.⁷² These cell lines depleted of mtDNA with EB have been developed as an important model in GSIS that is both reproducible and reversible.

The ethidium bromide technique has been used in a number of cell types demonstrating the critical importance of OXPHOS function in glucose-stimulated insulin secretion. The mouse pancreatic mtDNA knockout beta-cell line (rho0 MIN6 with no mtDNA) was found to lose the ability to secrete insulin in response to glucose, while MIN6 cells (repopulated with mtDNA) regained glucose-stimulated insulin secretion. Nuclear DNA transcription was not affected as evidenced by the continued transcription of insulin and succinate dehydrogenase in the knockout cells.⁷¹ Work with the MIN6 cell line has also shown that sulfonylurea drugs, which stimulate insulin secretion

via the inhibition of K_{ATP} channels, have impaired action with mtDNA depletion, suggesting that intracellular ATP is important in sulfonylurea signaling.⁷⁷

In another study, mtDNA-depleted rho(0) human hepatoma cell line (SK-Hep 1) showed that stimulation with glucose was unable to hyperpolarize the mitochondrial membrane potential.⁷⁸ The rhea(0) cell line (also mtDNA depleted) was found to have decreased intracellular ATP and glucose-stimulated ATP.⁷⁹ Similar results were obtained with cultures of the bHC9 pancreatic beta-cell line depleted with EB (0.4 ug/mL). This study showed the overall mitochondrial function was reduced and specifically transcription rates were reduced by 10-20 percent. This decrease was not reflected in nuclear transcription as evidenced by the transcription of insulin, glucokinase, and beta-actin. No abnormalities were found in other organelles such as the Golgi complex, nuclei, endoplasmic reticulum, or secretory granules. The activities of gluco- and hexokinase were also unchanged. Two days after the removal of EB, translation returned to normal and in seven days insulin secretion returned to normal.⁸⁰ Similar results have been obtained using rodent beta-cell line (INS-1 p^o lacking mtDNA) with 0.4 ug/mL EB for 3-12 months. This study also demonstrated that insulin secretion was not just an exocytotic process from the Golgi complex, but rather a result of mitochondrial function.⁷²

Taken as a whole, from the earlier experiments with specific inhibitors of mitochondrial complexes to more specific intercalation with EB inhibition of mitochondrial complex transcription, to more recent experiments with lower levels of EB that result in the temporary loss of mtDNA transcription, a bigger picture emerges as to the direct relationship of mtDNA-driven OXPHOS in the functioning of insulin release. Earlier experiments with specific mitochondrial complex inhibitors, although successful in demonstrating the relationship between mitochondria and GSIS, were not definitive. The EB studies supported the hypothesis of mitochondria as a key factor in GSIS, but at high levels EB also led to irreversible damage. The latest work using lower levels of EB demonstrates dysfunction directly related to the

decrease in mtDNA, which is reversible as mitochondrial transcription returns, furnishing GSIS. So, the mitochondria appear to be responsible for GSIS more by means of the mtDNA transcription of complexes than by nuclear transcription or other organelle function.

MtDNA Depletion and Glucose Transporters

A major characteristic of type 2 diabetes is insulin insensitivity due to dysfunction of glucose transporters in muscle and adipose tissue.⁸¹ Although decreases in mtDNA copy number in beta cells have demonstrated a decrease in GSIS, mtDNA depletion has also been shown to effect the expression of glucose transporters. Park et al⁷⁸ used an ethidium bromide mtDNA-depleted human hepatoma cell line (SK-Hep1) to analyze the mRNA levels of glucose transporter isoforms, GLUT-1, -3 and -4. The expression of all the glucose transporter isoforms was down-regulated in response to mtDNA depletion. GLUT-3 and -4 were expressed at lower levels than GLUT-1. This study links the reduction of mtDNA copy number to the decreased expression of nuclear DNA-encoded glucose transporters.

Human Studies of mtDNA Depletion

Further evidence of the essentiality of the mitochondria in the pathogenesis of type 2 diabetes in terms of insulin secretion and glucose uptake can be seen in a study of 55 patients in which peripheral blood leukocytes were analyzed for mtDNA content. These type 2 diabetic patients were found to have 25-35 percent lower mtDNA quantity, measured by Southern blot hybridization, as compared to controls. MtDNA content did not correlate with age, body mass index, duration of diabetes, or hemoglobin A1c (HBA1c) levels. Peripheral blood leukocyte mtDNA has been found to have good correlation with the mtDNA levels of the liver and muscle.⁸² More striking was the analysis of 23 prediabetic patients who converted to diabetes within two years. Prediabetic patients were found to have a significant decrease in mtDNA before the onset of disease as compared to controls.⁸²

The findings from type 2 diabetic and prediabetic patients are supported by earlier work that analyzed subtraction libraries (libraries indexing differentially expressed genes obtained through extractive hybridization) of skeletal muscle from type 2 diabetics and found an approximately 50-percent decrease in the mtDNA copy number.⁸³ Studies of healthy young men have demonstrated that mtDNA content in peripheral blood leukocytes have a direct relationship to fat and carbohydrate oxidation rate.⁸⁴ MtDNA copy levels were not directly correlated to insulin resistance and in another study of healthy young men an inverse relationship was found between mtDNA and both insulin resistance and secretion, indicating a possible compensatory response to insulin resistance.⁸⁵ These studies demonstrate that the quantitative decrease in mtDNA not only precedes the onset of diabetes, but also that the decrease is due to an underlying increase in oxidative stress. This presents a rationalization for the observed benefit of antioxidants in type 2 diabetes.

Treatment Strategies CoQ10

Although most of the focus has been placed on the qualitative (genetic) aspects of type 2 diabetes pathogenesis, the information presented shows that mitochondrial dysfunction leading to diabetes is somewhere along a continuum from increased oxidative stress to heterogeneous and homogeneous disease. Treatment strategies that focus on decreasing oxidative stress as well as increasing mitochondrial function might present important options. One treatment agent that might have clinical significance is coenzyme Q10 (CoQ10). A controlled study of trans-fatty acid fed rabbits found administration of CoQ10 (2 mg/day) for eight weeks produced a significant (15%) reduction in fasting and two-hour postprandial plasma insulin level (44%). Triglycerides along with fasting and two-hour postprandial glucose levels showed a significant decrease, while HDL increased.⁸⁶ The clinical benefit to patients with mitochondrial myopathies, MELAS, and other mitochondrial mutations employing CoQ10 has been evident in a number of therapeutic trials.^{12,87-94}

Early uncontrolled clinical studies of diabetic patients have demonstrated benefit from ingestion of CoQ7 (120 mg/day, readily transformed to CoQ10) for 2-18 weeks. Of the 39 patients, 12 patients reduced blood sugar by 20 percent and 12 by 30 percent, while ketone bodies were reduced by 30 percent in 13 patients.⁹⁵ In another study, 15 diabetic patients receiving CoQ10 (60 mg/day) were found to have an improvement in blood glucose and insulin synthesis and secretion.⁹⁶

Case studies of patients with maternally-inherited type 2 diabetes mellitus (often expressing the type 2 phenotype, readily identified through molecular screening of mtDNA and often associated with hearing loss) have reported success in relieving some of the neuromuscular complications as well as insulin edema associated with this disorder when using CoQ10.⁹⁷⁻⁹⁹ In a case study, a 35-year-old patient diagnosed with MELAS and diabetes mellitus due to a mitochondrial mutation (bp3234) tRNA^{Leu (UUR)} had a sudden onset of high blood glucose and convulsions. Treatment with CoQ10 (160 mg/day) yielded increased C-Peptide Response to Glucagon Stimulation – from 2.95 ng/mL before treatment to 4.16 ng/mL after six months of treatment. The patient was able to discontinue insulin and shift to oral hypoglycemic agents.¹⁰⁰

Twenty-eight patients with maternally-inherited diabetes mellitus and deafness syndrome possessing the most common mitochondrial mutation (bp3234) tRNA^{Leu (UUR)} were given 150 mg CoQ10/day (Neuquinon) for three years in an open trial. The treatment was found to prevent progressive insulin secretion defects, exercise intolerance, and hearing loss. Insulin secretory capacity of the beta cells as measured by C-Peptide Response to Glucagon Stimulation steadily rose: 17 percent, from base line (2.25) to two years (2.53), and three years (2.70). The control group steadily deteriorated 26 percent, from base line (2.0 ng/mL) to two years (1.78 ng/mL), and three years (1.48 ng/mL). It is interesting to note that CoQ10 had no short-term effect on insulin secretory capacity or clinical symptoms one to three months after beginning the trial.¹⁰¹ This trial suggests the lack of short-term effect of CoQ10 in alleviating symptoms.

The lack of short-term effect can be seen in another controlled study of 23 type 2 diabetic patients supplemented with 100 mg CoQ10/day (Bio-Quinone[®]), with the concomitant use of sulfonylurea drugs. No effect on blood glucose or HbA1c was found after six months of therapy.¹⁰² Similar results were reported by Silvestre-Aillaud et al¹⁰³ who found no significant difference in insulin secretion or insulin sensitivity after six months of a combination of CoQ10 (150 mg/day) and L-carnitine (2 g/day) for maternally-inherited type 2 diabetes mellitus. Although insulin secretion was not effected, there was an improvement of oxidative metabolism as evidenced by normalization of phosphocreatine/inorganic phosphate ratios.

In summary, although sufficient time is a critical factor for therapeutic success, CoQ10 seems more effective if treatment is begun early in the disease process. For example, in the case study by Silvestre-Aillaud et al,¹⁰³ the patient was age 40 with 74 percent of muscle cells showing mtDNA mutation and profound beta-cell dysfunction leading to a classic insulin-dependent diabetic picture. Higher dosages might give a better response in a shorter time. These studies also support the expansion of clinical use of CoQ10 for type 2 diabetic cases not maternally inherited or due to genetic defects.

Dietary Fats

Human consumption of dietary trans-fatty acids (TFAs) from partially hydrogenated vegetable oils has been estimated at three percent in the United States.¹⁰⁴ The concentration of TFAs, analyzed in eight patients with uncontrolled diets, was estimated by Ohlrogge et al¹⁰⁵ to be 2-6 percent in adipose and 1-3 percent in the liver. Earlier studies by Johnston et al¹⁰⁶ found trans-fatty acid levels of 2-12 percent in adipose and 4-14 percent in liver. Diets high in TFAs are thought to change the composition and decrease fluidity of membrane phospholipids,¹⁰⁷ which has a detrimental effect on the function of various membrane-associated enzymes and receptors.¹⁰⁸ There is evidence that TFAs have a lower rate of oxidation by mitochondria. This decrease in oxidation may be

due to a direct effect on the functioning of complex V or other complexes of the electron transport chain.

Dietary fats have been associated with both insulin resistance and insulin sensitivity. Saturated fatty acids are associated with insulin resistance while medium and long-chain fatty acids (especially omega-3) are associated with increased insulin sensitivity.¹⁰⁹ Previous studies have shown an inverse relationship between vegetable fats and risk of diabetes.¹¹⁰ Many vegetable polyunsaturated fats can also contain TFAs that can confound results. A prospective study of 84,204 healthy women nurses age 34-59 has found an increased risk for type 2 diabetes associated with ingestion of TFAs, when the data was adjusted for trans-fatty acids as compared to the other fatty acids.¹¹¹ The intake of TFAs was positively associated with risk of diabetes, while omega-3 and -6 fatty acids were inversely associated with risk. The data suggests that patients who replace two percent of the energy from trans-fatty acids with polyunsaturated fatty acids could decrease the risk for type 2 diabetes by as much as 40 percent.¹¹¹

Mitochondria have been shown to reflect changes in fatty acid content when test animals are fed trans-fatty acids. Rats have been found to replace 15-19 percent of the mitochondrial membrane content with TFAs when fed diets of 15-25 percent partially hydrogenated arachis oil (a deep sea fish) for ten weeks.¹¹² The fact that diets high in TFAs lead to changes in the bilipid membrane of the mitochondria raises the possibility for TFAs to inhibit mitochondrial-ATP production via inhibition of complex V (due to decreased membrane fluidity).

Schrijver et al¹¹³ found liver mitochondria of rats fed a diet of partially hydrogenated soybean oil (with 45-percent trans-octadecenoic acids (t18:1) with an ester concentrate of trans-fatty acids containing 52-percent trans, trans-octadecenoic acids (t,t18:2)) resulted in a significant decrease in the respiratory function of liver mitochondria. The decrease was inversely proportional to the degree of t,t18:2 incorporation in the liver mitochondria. A dietary threshold level greater than 2.5 percent for t,t18:2 TFAs resulted in a reduction ADP/oxygen ratio, ATP synthesis, and

state 3 and state 4 respiration oxygen uptake. The ADP/oxygen ratio, as well as state 3 and state 4 respiration, are all various ways to measure mitochondrial function. ADP/oxygen measures mitochondrial respiration looking at the extra atoms of oxygen consumed by a known amount of ADP. State 3 respiration is a measure of the active state of respiration where ADP is phosphorylated to ATP, while state 4 is a measure of respiration where no phosphorylation occurs due to lack of ADP or phosphate acceptors. Whether the decrease was an artifact of a change in the membrane fluidity was not determined.

Rats fed 20-percent (w/w) partially hydrogenated Norwegian capelin oil (HCO) for 10 weeks were found to have a significant decrease (13-20%) in ATP synthesis rate and ADP/oxygen ratio in cardiac mitochondria as compared to rats fed peanut oil.¹¹⁴ This finding corresponds to similar results obtained by rats fed either 10-percent sunflower oil or 10-percent hydrogenated oil containing 28-percent 18:1 trans fatty acids for a period of 60 days. Myocardial tissues from rats fed the TFAs diet were found to have a significant six-percent drop in mitochondrial respiratory activity with a 12-percent drop under anoxic conditions when compared to controls.¹¹⁵ Mixed TFA diets (39% t18:1, 0.6% c,t,t,c18:2, and 0.5% t,t18:2 w/w) fed to rats for nine weeks were also found to lead to 13-percent decrease in mitochondrial cytochrome c-oxidase activity as compared to cocoa butter and low-linoleic-acid olive oil diets.¹¹⁶ Rumenic acid (c-9, t-11-c18:2) comprises 75-90 percent of the conjugated linoleic acid found in dairy products. *In vitro* studies of rat liver mitochondria incubated with rumenic acid for 20 minutes demonstrated a decreased state 3 respiration by 50 percent and uncoupled respiration by 30 percent.¹¹⁷

These experiments have shown that TFAs have an overall lower rate of mitochondrial oxidation as compared to cis-fatty acid configurations. Whether this decrease is a consequence of a reduction in membrane fluidity, which in turn limits the functioning of complex V, or due to a decline in the other complexes in mitochondrial oxidation, or a combination of the two must still be studied. Work with complex V in experimental

models of diabetes demonstrates a possible avenue for treatment.

One factor in a treatment strategy should focus on increasing the efficiency of complex V by increasing the fluidity of the inner mitochondrial membrane. Complex V physically functions much like a turbine in a dam and uses the potential energy pumped into the membrane space via the other complexes to create ATP. As the protons rush back into the inner mitochondrial membrane along the proton gradient, ADP couples with phosphate to form ATP.

Selective breeding of a non-obese rat with maturity-onset hyperglycemia, which mimics human type 2 diabetes, produced the BHE/Cdb strain of rat. The BHE/Cdb strain has been found to have two mutations in the mitochondrial complex V. One of the mutations is located in the proton channel and the other in subunit 6 (also known as subunit a) of the mitochondrial complex. These rats develop hyperglycemia and weakened glucose tolerance as they mature and become adults.¹¹⁸⁻¹²⁰ The defect is passed through the genetic line of the mother, much like maternally-inherited diabetes mellitus.¹²¹ One intriguing aspect of this strain of rats focuses on a simple amino acid substitution – serine for threonine – in the mtDNA of the anchoring subunit 6, part of the complex V (F_1F_0 ATPase). This simple substitution has been suspected in the conformation change of the secondary protein structure from that of a pleated sheet to an amino acid loop.¹²² The F_0 portion of the F_1F_0 ATPase is imbedded in the inner mitochondrial membrane where it must rotate in relation to the matrix (F_1 portion).^{123,124}

Changes in membrane fluidity have been found to influence the F_0 portion of complex V. Studies have shown that rats fed hydrogenated coconut oil have a decrease in the coupling of ATP with pyruvate and succinate substrates.¹²⁵ A comparison was made between Sprague-Dawley and BHE/Cdb rats fed hydrogenated coconut oil (high in TFAs), corn oil (high in omega-6 fatty acids), or menhaden oil (high in omega-3 fatty acids). The study found that, while the coconut oil-fed BHE/Cdb rats had lower ADP/oxygen ratios when compared to the Sprague-Dawley rats, the ADP/oxygen ratio was equal when animals were fed the

menhaden fish oil.¹⁰⁷ Thus, changing the dietary fat source to a more viscous hydrogenated form (and higher TFAs) results in a significant decrease in ATP production for energy. While TFAs have been shown to decrease energy and complex V function, studies have shown that rats fed an omega-3 rich diet containing sardine oil demonstrated increased activation of complex V.¹²⁶

In summary, these studies appear to support the use of omega-3 oils and the elimination of TFAs and saturated fat in type 2 diabetes.

Conclusion

This article demonstrates that type 2 diabetes is not merely a disease of insulin insensitivity or lack of insulin release but may be a global dysfunction of the mitochondrial energy system. The unique role of mitochondria in the genetics and metabolism associated with the pathophysiology of type 2 diabetes has been reviewed. The basic structure and function of the mitochondria illustrates its importance, not only in insulin secretion, but also in aspects of mitochondrial and nuclear transcription affecting the entire body. Damage to mtDNA has been shown in certain situations to result in the type 2 diabetes phenotype. The expression of this phenotype seems dependent on a number of critical thresholds associated with mutations and tissue OXPHOS capacity. Another aspect of dysfunction is associated with the quantitative reduction in mtDNA copy number. Decrease of mtDNA can be considered a causal factor in type 2 diabetes. This reduction in mtDNA can predict the onset of type 2 diabetes in prediabetics and is an important factor in glucose-stimulated insulin release. Although reduction of mtDNA is a critical factor in type 2 diabetes pathology, the question remains as to the nature of the original insult. The role of dysregulated ROS, identified as the primary offending agent, raises the question regarding which external protoxins or environmental chemicals (with actions such as MPTP and streptozotocin) might yet be isolated in connection with type 2 diabetes.

Properly designed studies are needed to determine whether a diet extremely low in TFAs is capable of improving or reversing type 2

diabetes. Likewise, a long-term study is needed to demonstrate whether CoQ10 can improve or reverse type 2 diabetes not caused by genetic mutation of mtDNA. Studies are also needed to examine the prevention of diabetes in patients found to be at risk due to decreased content of mtDNA in peripheral blood leucocytes (25-30% decrease).⁸² From an understanding of mitochondrial dysfunction in type 2 diabetes a number of treatments become logical supports. Two treatment strategies should be considered. First, remove offending toxins; for example, the elimination of dietary TFAs. Although controlled trials have not been conducted as to the efficacy of the elimination of TFAs, the evidence so far supports elimination. Second, establish normal cellular function. CoQ10 is a critical part of the ETC and an antioxidant. Other antioxidants (e.g., vitamin E, bioflavonoids, and lipoic acid) are known to reduce ROS in the mitochondria.¹²⁷⁻¹³⁰

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