Mitochondrial Dysfunction in Diabetes Mellitus

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ABSTRACT

This review discusses the hypothesis that mitochondrial dysfunction plays a role in the pathogenesis of the most common form of diabetes, type II diabetes mellitus. Mitochondrial mutations have been linked to the development of diabetes mellitus as part of several rare syndromes, accounting for approximately 1.5% of all cases of the disease ("classic" mitochondrial diabetes). The characteristics of classic mitochondrial diabetes are intermediate between those of type I and type II diabetes, more closely resembling the latter. By studying the biochemical, cellular, and physiologic consequences of mitochondrial DNA mutations that cause classic mitochondrial diabetes, we may also gain important insights into the pathogenesis of type II diabetes mellitus. Individuals with classic mitochondrial diabetes exhibit a variety of defects in mitochondrial electron transfer enzyme activities. Complex I and Complex IV activities in skeletal muscle are almost universally decreased in mitochondrial diabetics compared with control individuals. The major physiologic abnormality in classic mitochondrial diabetes is delayed and insufficient insulin secretion in response to a glucose load. Insulin resistance is less commonly observed in these patients. The link between mitochondrial function and insulin secretion is supported by cellular studies in which introduction of inhibitors of oxidative phosphorylation or depletion of mitochondrial DNA markedly impairs glucose mediated insulin secretion from pancreatic β-cells. Evidence for mitochondrial dysfunction in the common form of type II diabetes includes excessive free radical levels in the plasma of diabetics, increased reactive oxygen species, and decreased ATP synthase activity in cybrids constructed from mitochondria of diabetic patients, and maternal inheritance of the disease. By using the occurrence of diabetes in rare mitochondrial syndromes as an example, evidence supporting a relationship between mitochondrial dysfunction and the common form of type II diabetes is discussed. Mitochondrial function may represent a novel area for the development of therapeutic and diagnostic strategies for type II diabetes mellitus. Drug Dev. Res. 46:67–79, 1999. © 1999 Wiley-Liss, Inc.

Key words: insulin secretion; oxidative phosphorylation; mitochondrial diabetes; type II diabetes; pancreatic beta cell

INTRODUCTION

Mitochondrial dysfunction has been linked with several uncommon syndromes that are characterized by chronic neurodegenerative disease, myopathy, and a variety of other symptoms. It has become evident over the past decade that some of these rare mitochondrial diseases are also linked with diabetes mellitus. As a result, there is growing interest in defining whether the mitochondria play a role in the pathogenesis of the most common form of diabetes, namely type II or non–insulin-dependent diabetes mellitus (NIDDM). Such a finding would not only greatly improve our understanding of the disease mechanism but would also present an entirely new strategy for therapeutic intervention and diagnostic development. To date, mitochondrial dysfunction in diabetes has been identified in individuals with mutations in mitochondrial genes. As will be discussed, mitochondrial dysfunction may also arise from nuclear mutations in genes encoding mitochondrial enzyme subunits or factors that regulate the expression of mitochondrial proteins. Alternatively, mitochondrial dysfunction may arise...
from an overall decline in cellular content of mtDNA, or from metabolic factors that regulate mitochondrial function secondarily. In this overview, we will discuss the characteristics of rare classic mitochondrial diabetes, the cellular and molecular mechanisms involved in normal glucose homeostasis and in classic mitochondrial diabetes, and the potential roles of mitochondria in the common form of NIDDM.

SUBTYPES OF DIABETES MELLITUS: A BRIEF OVERVIEW

Type II, or NIDDM is the most common form of diabetes, affecting an estimated 16 million people in the United States alone. In fact, NIDDM is the most common type of diabetes worldwide, accounting for nearly 90% of all cases of diabetes mellitus (Fig. 1). Type I, or insulin-dependent diabetes mellitus, accounts for approximately one tenth of cases, whereas proven cases of mitochondrial diabetes (i.e., cases in which specific mitochondrial mutations have been identified; “classic” mitochondrial diabetes) account for approximately 1.5% of all cases of diabetes [Gerbitz et al., 1995; van den Ouweland et al., 1995]. Both NIDDM and mitochondrial diabetes are heterogeneous in their clinical presentations. However, certain generalizations can be made for the purpose of comparison. The hallmarks of NIDDM are its onset in middle aged, obese individuals; the presence of peripheral insulin resistance and impaired insulin secretion; absence of ketosis; and a high (>85%) concordance rate between identical twins. Type I diabetes typically has its onset in childhood or young adulthood, and is characterized by absolute insulin deficiency and a tendency to develop ketosis. These individuals are usually thin; the concordance rate is <40% for identical twins. Mitochondrial diabetes presents as an intermediate syndrome, more closely resembling type II diabetes in most patients. The onset of mitochondrial diabetes is generally in adults before the age of 40 who are typically not obese. Ketosis occurs infrequently. However, as will be discussed below, insulin secretory capacity tends to decline more rapidly than in NIDDM, such that a larger proportion of mitochondrial diabetics ultimately require treatment with insulin. Mitochondrial diabetics usually do not present with the human lymphocyte antigen associations or islet cell antibodies that are observed in type I diabetics. The complications of mitochondrial diabetes are similar to those of type I and type II diabetes mellitus. However, the precise incidence and onset of complications is difficult to establish, because most reports of mitochondrial diabetes mellitus have not discussed complications. Furthermore, identification of symptoms that are clearly secondary to diabetes is confounded by the coexistence of neurologic and muscular symptoms related to the underlying mitochondrial syndrome. On the basis of reports that have discussed diabetic complications [e.g., Suzuki et al., 1994; Kishimoto et al., 1995], retinopathy, nephropathy, and sensorineural hearing loss occur frequently. This observation illustrates that angiopathy is a common complication of mitochondrial, type II, and type I diabetes.

Mitochondrial diabetes mellitus can be further subdivided based on (1) clinical characteristics and (2) the specific mutation(s) involved. Because the phenotype associated with a given genotype can be highly variable and can change with time, characterization by clinical presentation is dynamic and can be a bit confusing. For example, an individual with the tRNA$^{	ext{Leu}}$(UUR) 3423 mutation may present with diabetes alone, diabetes and deafness, or the full-blown mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome [van den Ouweland et al., 1995; Gerbitz et al., 1995; Rotig et al., 1996]. Patients may progress from diabetes to diabetes and deafness to MELAS. Thus, the same genotype can present simply as diabetes, as maternally inherited diabetes and deafness (MIDD), or as MELAS. Moreover, the same individual may progress through multiple diagnoses as additional symptoms develop. Conversely, an individual diagnosed with MIDD could have the tRNA$^{	ext{Leu}}$(UUR) 3423 mutation, the 10.4 kd mtDNA deletion described by Ballinger et al. [1992], or other mtDNA changes. In short, mitochondrial diabetes is a complex and variable group of diseases. If mitochondrial dysfunction also plays a role in the more prevalent form of common NIDDM, there is no reason to predict that the dysfunction will be homogeneous in all cases of NIDDM.

GENETICS OF MITOCHONDRIAL DNA

The occurrence of diabetes in individuals with known mtDNA mutations may provide important clues about the pathogenesis of NIDDM. Specifically, under-
standing the relationship among the mtDNA changes, mitochondrial function, and glucose homeostasis in mitochondrial diabetes may guide us in the search for the underlying defect(s) in NIDDM. Inherent in this premise is the idea that mitochondrial dysfunction resulting from a variety of causes (primary genetic defects, secondary to metabolic abnormalities) may affect insulin secretion or insulin action. The present discussion will focus first on the genetics of mitochondrial diabetes and NIDDM, and then on the biochemical and physiological correlates of mitochondrial function in diabetes mellitus.

Human mitochondrial DNA (mtDNA) is composed of a 16,569-bp double-stranded circular DNA molecule that is present in multiple copies per mitochondrion. This genome encodes 13 proteins (all of which are subunits of mitochondrial electron transfer complexes), 22 tRNAs, and 2 rRNAs [Anderson et al., 1981; Wallace, 1994]. All of the other subunits of the electron-transfer complexes are encoded by the nuclear genome, as are the transcription factors, replication factors, DNA polymerase, and regulatory factors for mitochondrial protein synthesis [Clayton, 1991, 1992]. Because most cells contain dozens to hundreds of mitochondria, each mitochondrial gene is represented many times in a cell [Wallace, 1992]. Moreover, alternative or mutant forms of a given gene can be present in any proportion from 0 to 100%, a condition termed heteroplasmy. Inheritance of the mitochondrial genome is maternal in humans and other mammals. Because mtDNA lacks protective histones, has minimal repair mechanisms, and is present in a free radical-rich environment, the spontaneous mutation rate is high relative to nuclear DNA [Richter et al., 1988; Wallace, 1994]. This finding is particularly true in the pancreatic islet, where levels of antioxidant enzymes are significantly lower than in any other tissue examined [Lenzen et al., 1996]. Furthermore, it is likely that mtDNA mutations accumulate with age. Some mtDNA mutations may ultimately cause mitochondrial dysfunction (and a disease phenotype) if the amount of mutant mtDNA relative to wild-type mtDNA (the level of heteroplasmy) increases to a high enough level. Individuals who begin life with a relatively high level of heteroplasmy at specific sites are, therefore, more likely to achieve a threshold level required for expression of a disease phenotype.

Hence, the typical mitochondrial disease shows a maternal inheritance pattern, can have a variable phenotype, and has a chronic progressive nature. These same features are, in fact, observed in many individuals with NIDDM. It is well-established that development of NIDDM involves a strong genetic component, as indicated by a concordance rate in identical twins that exceeds 70% [Rotter et al., 1990]. Moreover, children of one type II diabetic parent have a 30–50% chance of developing the disease, whereas the risk for offspring of two diabetic parents approaches 70% [Groop, 1997]. In Caucasian populations, transmission of type II diabetes is predominantly maternal, as illustrated by similar results in studies of Finnish [Groop et al., 1996], French [Thomas et al., 1994], and British [Alcolado and Alcolado, 1991] populations.

Extensive efforts to identify the relevant genes in NIDDM have not revealed a clear-cut genetic basis for the majority of type II diabetics. Of note, virtually all of the searches for causative genes have been directed at the nuclear genome. Investigations of the mitochondrial genomes of type II diabetics have involved only analysis of known mutation “hot spots,” or have used restriction fragment length polymorphisms, which can miss important alterations. Given the intriguing similarity of inheritance patterns in NIDDM and mitochondrial diabetes, a reasonable argument can be made to further the search for mitochondrial genetic defect(s) in NIDDM.

**BIOCHEMICAL AND PHYSIOLOGIC CORRELATES IN MITOCHONDRIAL DIABETES**

To better understand the potential role of mitochondrial dysfunction in diabetes, it is informative to consider the biochemical and physiologic consequences of known mitochondrial mutations that are associated with diabetes. This may allow us to predict which aspects of mitochondrial function, whether encoded by the mitochondrial genome or occurring as secondary phenomena, contribute to the pathogenesis of NIDDM and its complications. A brief overview of the proposed functions of mitochondria in glucose homeostasis will be considered first.

**Cellular mechanisms in glucose homeostasis**

Insulin is secreted from pancreatic β-cells in response to glucose and a variety of other secretagogues [Meglasson and Matschinsky, 1986; Liang and Matschinsky, 1994; Newgard and McGarry, 1995; Prentki, 1996; DeFronzo, 1997]. Glucose is the most important of these secretagogues in vivo. As the biochemical mechanisms coupling glucose uptake by the β-cell to secretion of insulin are being elucidated gradually, the importance of mitochondria for this process is emerging.

According to the generally accepted paradigm of glucose-mediated insulin secretion, the initial step is uptake of glucose into the β-cells by means of the GLUT2 glucose transporters (Fig. 2). Uptake significantly exceeds glucose utilization and is, therefore, not rate limiting for the sequence of events that triggers insulin release [Newgard and McGarry, 1995; Matschinsky, 1998; Matschinsky et al., 1998]. Rather, it is the subsequent phosphorylation of glucose to glucose-6-phosphate (G6P) that seems to define the setpoint at which secretion is initiated. Islets contain both a low K_m (hexokinase I) and a high K_m (glucokinase = hexokinase IV) glucose phosphorylating activity. The K_m
of glucokinase is 6–11 mM, whereas that of hexokinase I is 10–100 µM. Furthermore, hexokinase I is inhibited by its product, G6P, whereas glucokinase is not. The majority of glucose phosphorylating activity in β-cells is accounted for by the high K_m glucokinase. The low K_m hexokinase I is proposed to be inactive in the islets due to inhibition by G6P. As a result, insulin secretion normally occurs when the blood glucose begins to rise above the physiological level of ~5.5 mM.

Glucokinase is bound to the outer surface of the mitochondria in β-cells through its interaction with the protein porin (also called VDAC or voltage-dependent anion channel) [Sener et al., 1986; Malaisse-Lagae and Malaisse, 1988; Muller et al., 1994; Rabuazzo et al., 1997]. This situation is analogous to the interaction of hexokinase II with mitochondria in liver and skeletal muscle, and hexokinase I with mitochondria in liver [Weiler et al., 1985; Adams et al., 1988; Gerbitz et al., 1996]. Because porin is associated with the adenine nucleotide translocator, binding of GK to the pore may facilitate delivery of oxidatively produced ATP to the enzyme (which preferentially uses ATP produced by the mitochondria [Rasschaert and Malaisse, 1990]). Delivery of ADP back to the mitochondria for resynthesis of ATP by Complex V may also be a function of this association [Laterveer et al., 1994]. The importance of glucokinase as the glucose sensor is illustrated by the development of diabetes in individuals who have mutations of the glucokinase gene [Froguel et al., 1993; Bell et al., 1996]. Maturity onset diabetes of the young (MODY) is a form of diabetes mellitus that resembles NIDDM clinically, but has its onset before the age of 25, is generally milder, and has an autosomal dominant mode of transmission. At least three distinct mutations have been identified in MODY families [Bell et al., 1996]. MODY 2 is characterized by mutations of the glucokinase gene, resulting in a predicted 50–100% decrease in glucokinase activity and impaired insulin secretion [Hattersley et al., 1992; Froguel et al., 1993]. The occurrence of diabetes in heterozygous individuals who have some residual glucokinase activity underscores the role of glucokinase as the rate limiting glucose sensor of the β-cell.

After glucose phosphorylation, the subsequent fate of G6P is almost entirely metabolism through the glycolytic pathway, because the pentose phosphate shunt is relatively inactive in pancreatic β-cells [Ashcroft et al., 1972], and glycogen synthesis accounts for no more than 7% of glucose flux [Meglasson and Matschinsky, 1986]. The glycolytic pathway distal to G6P seems particularly important for insulin secretion with regard to the production of NADH [MacDonald and Fahien, 1990; Dukes et al., 1994], which is efficiently shuttled from the cytosol to the mitochondria. There, it enters the electron transport chain at complex I and fuels oxidative production of ATP. The next clear-cut correlation between cellular metabolism and insulin secretion.

### Table 1. Summary of Biochemical Defects in Tissues of Patients with Mitochondrial Diabetes

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Tissue</th>
<th>Citrate synthase</th>
<th>Complex I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Skeletal muscle</td>
<td>↔</td>
<td>↓</td>
<td>nd</td>
<td>↔</td>
<td>↓</td>
<td>nd</td>
<td>van den Ouweland et al., 1992</td>
</tr>
<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Skeletal muscle</td>
<td>nd</td>
<td>↓</td>
<td>↓</td>
<td>↔</td>
<td>↔</td>
<td>↓</td>
<td>Suzuki et al., 1994b</td>
</tr>
<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Skeletal muscle</td>
<td>↓</td>
<td>nd</td>
<td>↓</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>Schulz et al., 1993</td>
</tr>
<tr>
<td>10.4-kd deletion</td>
<td>Skeletal muscle</td>
<td>↓</td>
<td>nd</td>
<td>↓</td>
<td>↔</td>
<td>↓</td>
<td>nd</td>
<td>Ballinger et al., 1992, 1994</td>
</tr>
<tr>
<td>5778-bp deletion</td>
<td>Skeletal muscle</td>
<td>↓</td>
<td>nd</td>
<td>↓</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>Kobayashi et al., 1997</td>
</tr>
<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Pancreatic islet</td>
<td>↓</td>
<td>↑</td>
<td>nd</td>
<td>↓</td>
<td>↓</td>
<td>nd</td>
<td>Hinokio et al., 1995</td>
</tr>
<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Skeletal muscle</td>
<td>↓</td>
<td>nd</td>
<td>↓</td>
<td>↔</td>
<td>↓</td>
<td>nd</td>
<td>Hanna et al., 1995</td>
</tr>
<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Cybrids</td>
<td>↓</td>
<td>nd</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>nd</td>
<td>Dunbar et al., 1996</td>
</tr>
<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Cybrids</td>
<td>nd</td>
<td>nd</td>
<td>↓</td>
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<td>nd</td>
<td>nd</td>
<td>Mariotti et al., 1994</td>
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<tr>
<td>tRNA^{+UUR}_{3243}</td>
<td>Cybrids</td>
<td>↔</td>
<td>nd</td>
<td>↓</td>
<td>nd</td>
<td>↔</td>
<td>↓</td>
<td>King et al., 1992</td>
</tr>
</tbody>
</table>

*↑, no change from controls; ↓, decreased relative to controls; ↔, increased relative to controls; nd = not determined.*
insulin secretion is the rise in the intracellular ATP:ADP ratio [Ashcroft et al., 1973; Longo et al., 1991; Erecinska et al., 1992], which triggers closure of the ATP-sensitive K$^+$ channel at the β-cell plasma membrane, resulting in depolarization of the cell. The increase in ATP:ADP after a glucose load is believed to be due to a rise in ATP of predominantly oxidative origin [Malaisse, 1992]. In fact, using a series of glycolytic inhibitors, Dukes et al. [1994] demonstrated that only oxidatively derived ATP could trigger closure of the K$^+$ channel in β-cells. This membrane depolarization leads to opening of Ca$^{2+}$ channels with influx of calcium to the cytosol. It is the rise in intracellular calcium that ultimately causes the exocytosis of insulin. Using β-cells in culture (HIT), Civelek and coworkers [1996a,b] have confirmed the proposed temporal relationship, establishing that glucose phosphorylation precedes the rise in ATP:ADP, which precedes the rise in intracellular calcium.

According to this model of glucose-mediated insulin secretion, the β-cell mitochondria play an important role in insulin release. The model is supported by cellular studies that demonstrate that manipulation of mitochondrial function can alter normal glucose homeostasis. At the cellular level, glucose-stimulated insulin secretion can be abrogated by a variety of metabolic inhibitors, including oligomycin, azide, antimycin A, rotenone, cyanide, and the uncoupler FCCP [MacDonald and Fahien, 1990; Kiranadi et al., 1991; Detimary et al., 1994; Dukes et al., 1994]. Using oligomycin to inhibit ATP synthase, our laboratory has established that the catalytic activity of ATP synthase is tightly coupled to insulin secretion, such that even a minor defect in the activity of the enzyme would be predicted to cause a similar impairment of glucose-stimulated insulin secretion (Fig. 3).

Similarly, decreasing all mitochondrial-encoded enzyme activities by depleting mtDNA eliminates glucose-stimulated insulin secretion. Soejima and coworkers [1996] used bis-4-piperidyl dichloride to deplete mtDNA from the mouse pancreatic β-cell line MIN6. These cells expressed no detectable mitochondrially encoded proteins, no cytochrome oxidase activity, and no glucose-stimulated insulin secretion. Tsuruzoe and coworkers [1998] performed a similar series of experiments using MIN6 cells that were depleted of mtDNA with ethidium bromide. Their cell line also lost the ability to secrete insulin or to increase intracellular ATP in response to glucose, but retained the ability to secrete insulin in response to sulfonylurea or KCl. Kennedy and colleagues [1998] treated the rat-derived INS-1 cell line with ethidium bromide to deplete the majority of mtDNA, with a similar loss of ATP and insulin responses to glucose. Our laboratory has taken a slightly different approach, by using the antiviral compound dideoxyctydine (ddC) to deplete mtDNA from INS-1 cells [Anderson et al., 1998]. Like the cells constructed by Kennedy and coworkers [1998], our ρ0 INS-1 cells retained normal basal insulin secretion, but failed to increase insulin secretion in response to glucose (Fig. 4). In contrast, a normal insulin secretory response to KCl was observed, suggesting that the insulin secretory machinery distal to the mitochondria was intact. Similarly, intracellular ATP
levels did not change in response to glucose in the \( \rho^0 \) INS-1 cells (Fig. 5). The shift from oxidative to glycolytic ATP production in this \( \rho^0 \) cell line was also demonstrated by an increase in lactate production by the \( \rho^0 \) cells compared with the parental INS-1 cells.

Thus, there is little doubt about the importance of normal mitochondrial function in glucose-stimulated insulin secretion. However, the role of mitochondria in glucose utilization, the other key component of glucose homeostasis, is not well understood. The occurrence of mild to moderate insulin resistance in some cases of mitochondrial diabetes suggests that mitochondrial function may be involved in insulin sensitivity [see below; van den Ouweland et al., 1992; Sue et al., 1993; Kanamori et al., 1994; Kishimoto et al., 1995]. Moreover, two separate studies have shown an increased incidence of mtDNA alterations in populations of patients with NIDDM or impaired glucose tolerance (which is characterized by insulin resistance) compared with individuals with normal glucose tolerance [Liang et al., 1997; Poulton et al., 1998]. The role of mitochondria in peripheral insulin sensitivity may relate to the interaction of hexokinase with the mitochondrial protein porin. As noted above, hexokinase associates with the mitochondria in skeletal muscle, resulting in activation of the enzyme [Weiler et al., 1985; Adams et al., 1988; De Vos et al., 1991] and facilitating delivery of ATP to the enzyme. The specific effects of mitochondrial mutations and mitochondrial dysfunction on the activity of hexokinase remain to be determined but may contribute to impaired insulin-mediated glucose utilization. In individuals with the common form of NIDDM, hexokinase activity in skeletal muscle was reported to be low [Vestergaard et al., 1995; Kruszynska et al., 1998] and failed to increase normally during a hyperinsulinemic clamp study [Kruszynska et al., 1998]. Although Simoneau and Kelley [1997] observed a slight increase rather than a decrease in hexokinase activity in NIDDM skeletal muscle, they documented an overall decline in oxidative enzyme activities relative to glycolytic activities. Although it is not yet clear whether such alterations in metabolism in NIDDM are primary or secondary events, these observations further illustrate a potential role for mitochondrial metabolism in peripheral glucose utilization.

**Biochemical abnormalities in mitochondrial diabetes**

As discussed in the preceding section, mitochondrial function is necessary for normal glucose-mediated insulin secretion as shown by studies of both isolated islets and cell cultures. Rare syndromes that are characterized by mtDNA mutations permit us to determine how dysfunction of various mitochondrial enzymes affect glucose homeostasis in vivo. On the basis of cellular studies, one would predict that virtually any defect that results in decreased mitochondrial ATP production in the pancreatic islet could disrupt normal insulin secretion.

Toward that end, several investigators have analyzed mitochondrial enzyme activities in skeletal muscle biopsies or fibroblasts of patients with mitochondrial diabetes and their family members. The most extensively studied mtDNA mutation associated with diabetes mellitus is the tRNA\(_{\text{leu(UUR)}}\) 3243 mutation. van den Ouweland and coworkers [1992] measured skeletal muscle mitochondrial enzyme activities in an individual from a family with maternally transmitted diabetes and deafness who harbored the mutation. Complex I and IV activities were lower than control values, whereas Complex III and pyruvate dehydrogenase were low normal and citrate synthase was normal. A similar study by Suzuki et al. [1994b] found normal Complex II, III, and IV activities but low Complex I activity in skeletal muscle biopsies from six patients with MELAS and diabetes associated with the tRNA\(_{\text{leu(UUR)}}\) 3243 mutation. In a case report, Schultz and colleagues [1993] found a similar decrease in Complex I activity in a patient with diabetes and deafness and the 3243 mutation associated with a syndrome more closely resembling Kearns-Sayre syndrome than MELAS. In a rare examination of islet cells, an autopsy study of the pancreas from a woman with diabetes mellitus and the 3243 mutation showed decreased cytochrome oxidase activity and elevated succinate dehydrogenase activity in the islets using histologic techniques [Kobayashi et al., 1997]. Studies of patients or of cybrid cells constructed from tissues of patients with the MELAS mutation, but who did not necessarily have diabetes, typically have shown defects in Complex I, Complex IV, or both.

**Fig. 5.** Effect of glucose on intracellular ATP levels in INS-1 cells and mtDNA-depleted (“rho0”) INS-1 cells. Cells were glucose starved for 1 h, then incubated with various concentrations of glucose for an additional hour. The cells were extracted with 5% tricarboxylic acid, and the ATP levels were measured using a luciferase assay.
[Chomyn et al., 1992; King et al., 1992; Mariotti et al., 1994; Brown and Wallace, 1994]. These defects have been attributed to a defect in mitochondrial protein synthesis [King et al., 1992; Brown and Wallace, 1994]. The relationship between heteroplasmy and the extent of the biochemical defect remains unclear.

Ballinger and coworkers [1992, 1994] studied a family with maternally inherited diabetes and deafness associated with a 10.4-kb mtDNA deletion. The proband had defects in the activities of complexes I–IV. Two brothers who were examined had defects in Complex III, and a daughter had defects in Complex III and Complex IV. In a second study of mtDNA deletion and diabetes, Hinokio et al. [1995] studied five patients with diabetic myopathy and nephropathy; all of whom harbored a 5778-bp deletion that included the coding regions for 4 ND subunits, COX III, and ATPase 6 and 8. Complex I activity in skeletal muscle biopsies was reduced in all five patients; Complex IV was reduced in one patient, but was normal in the other four; as were ATP synthase, succinate dehydrogenase, and succinate-cytochrome C reductase. A unique family with encephalomyopathy and diabetes mellitus associated with a tRNA glutamic acid mutation at position 14709 was reported by Hanna and coworkers [1995] to have a decrease in Complex I and IV activities, but normal nuclear-encoded citrate synthase and Complex II activities.

On the basis of these results, the most frequently detected biochemical defect in mitochondrial diabetes syndromes is a decrease in Complex I activity. Such a decline is likely to lead to a generalized decline in the overall efficiency of the electron-transport chain and decreased ATP synthesis, as would decreases in Complex III or IV observed in some patients. Thus, the common defects that one might predict from studies of mitochondrial diabetes are decreased ATP production and increased free radical production, due to inefficient functioning of the electron transport chain. Whether the observations regarding skeletal muscle enzyme activities are representative of the activities in pancreatic islets is unclear. Kobayashi’s autopsy study of the pancreas from a single individual with diabetes and deafness and the tRNA^leu^3243 mutation showed markedly decreased Complex IV activity in the islets by histochemical techniques [Kobayashi et al., 1997]. In addition, the level of heteroplasmy was much higher in the islets than in peripheral tissues (63% in islets compared with 8% in white blood cells), suggesting that defects measured in peripheral tissues may actually underestimate the magnitude of the defect in the islets.

**Physiologic abnormalities in mitochondrial diabetes**

Another approach to clarify the relationship between mitochondrial mutations and the diabetic phenotype has been to characterize the endocrine dysfunction of the affected individuals. As with the biochemical analyses, the majority of studies have involved individuals with the tRNA^leu^3243 (MELAS) mutation.

Sue and colleagues [1993] performed oral glucose tolerance tests (OGTT) on seven members of two families with maternally inherited diabetes and deafness. The four diabetic family members had normal or elevated fasting insulin levels, and a somewhat blunted rise in insulin in response to an oral glucose load. In a more extensive study of seven MELAS families, Suzuki and coworkers [1994b] analyzed insulin secretion and insulin sensitivity in 7 subjects with the mutation and symptoms of MELAS; 17 with the mutation but no neurological manifestations, and 11 without the mutation and with normal glucose tolerance. Of the mutant patients, 14 had diabetes mellitus, 3 had impaired glucose tolerance (IGT), and 7 had normal glucose tolerance. Although fasting insulin levels were not reported, 24-h C-peptide excretion was present but reduced, in order of severity, in diabetics, IGT, and nondiabetic MELAS individuals. Similarly, the rise in C-peptide in response to an intravenous glucose load was diminished in diabetics > IGT > MELAS with normal glucose tolerance. Again, nonmutant family members had a normal insulin response. Insulin sensitivity measured during an euglycemic clamp was normal in the four individuals who were tested. These investigators concluded that the mitochondrial tRNA^leu^3243 mutation is associated with a β-cell insulin secretory defect and that the severity of the defect is correlated with the clinical manifestation of diabetes. A study of 19 patients by Kadowaki et al. [1995] showed similar results, with low 24-h urinary C-peptide excretion and a subnormal rise in plasma insulin in response to a glucagon challenge.

van den Ouweland’s examination of a family with the tRNA^leu^3243 mutation and NIDDM reported results [van den Ouweland et al., 1992] similar to those reported by Sue et al. [1993], with normal or slightly elevated fasting insulin levels. Insulin levels during an OGTT were apparently normal in this family, although the timing and magnitude of the response are not reported [van den Ouweland et al., 1992]. Kishimoto et al. [1995] confirmed an insulin secretory defect in response to glucose in six affected patients, and they also detected moderate insulin resistance in one subject during euglycemic clamp. Finally, Oka and coworkers [1995] screened four diabetic pedigrees with 18 affected members. Their report highlights the variability in presenting phenotype, from mild NIDDM to insulin dependence, in individuals with the same tRNA^leu^3243 mutation. Their results generally indicate some preservation of insulin secretory capacity, but impaired response to a glucose load. Serial studies of one patient over a 5-year period documented the progressive decline in insulin secretory capacity that seems to typify diabetes mellitus associated with the position 3243 mutation.
Thus, all studies of the 3243 mutation have demonstrated a defect in insulin secretory capacity that seems to worsen with duration of diabetes. There is also some evidence for peripheral insulin resistance, based on the observation of elevated fasting insulin levels in some patients and abnormal responses in some euglycemic clamp studies that have been performed. It is possible that insulin resistance is a common feature of mitochondrial diabetes but that the typically cross-sectional design of most studies could not detect elevations of fasting insulin levels early in the disease. Specifically, because of the progressive nature of the insulin secretory defect in these patients, elevated fasting insulin may only be present very early in the natural history of the illness.

Diabetic individuals with a 10.4-kb mtDNA deletion exhibited clinical characteristics similar to those of patients with the tRNA^{lys} 3243 mutation, with low to normal fasting C-peptide levels and a blunted response to a glucagon challenge [Ballinger et al., 1992]. A patient with tRNA glutamic acid 14709 mutation with diabetes and myopathy had both decreased β-cell function and peripheral insulin resistance [Hanna et al., 1995]. A family with maternally inherited diabetes and myoclonus epilepsy and ragged red fibers syndrome (MERRF) associated with a tRNA^{lys} 8344 mutation has also been studied [Suzuki et al., 1994a]. Seven family members lacking the mutation had normal glucose tolerance; five of seven mutant family members were diabetic, one had impaired glucose tolerance, and one was normal. Insulin secretory capacity was decreased in the family members with the mutation.

The role of the respiratory chain in insulin secretion is also underscored by Suzuki’s recent report that coenzyme Q_{10} prevents progression of the insulin secretory defect in diabetics with MIDD associated with the tRNA^{lys} 3243 mutation (Suzuki et al., 1998). Moreover, the efficacy of coenzyme Q_{10} illustrates the potential therapeutic utility of agents that enhance mitochondrial function in diabetes.

Hence, there is excellent evidence that a variety of mtDNA mutations that result in generalized decreases in electron-transport chain activity cause β-cell secretory dysfunction and, at least in some cases, peripheral insulin resistance. In type II diabetes, the coexistence of peripheral insulin resistance and an insulin secretory defect is well established. It is also known that the insulin secretory defect gradually worsens with progression of the disease, ultimately leaving some patients with frank insulin deficiency and the need for insulin therapy. Modest pancreatic β-cell loss has also been reported in NIDDM [Westermark and Wilander, 1978; Clark et al., 1988; Volk, 1990], but the magnitude of this loss is unlikely to be sufficient to account for the decline in insulin secretion [DeFronzo, 1997]. This finding suggests that there is intrinsic dysfunction of the β-cells themselves. Although the extent of this dysfunction can be minimized with tight control of blood glucose, it cannot be completely normalized [reviewed in Rosetti et al., 1990; DeFronzo, 1997]. Similarly, peripheral insulin resistance can be improved, but not returned to normal, with tight glucose control [Garvey et al., 1985; Olefsky and Molina, 1990]. These observations suggest that the disease phenotype results from the complex interaction of metabolic factors and underlying biochemical defects. We postulate that mitochondrial dysfunction contributes to the underlying biochemical defects in the common form of NIDDM.

Evidence of mitochondrial dysfunction in common NIDDM

Little is known about the activities of mitochondrial enzymes in the tissues of individuals with NIDDM compared with individuals with normal glucose tolerance. It has not, therefore, been possible to address whether proven mitochondrial diabetes and NIDDM share common biochemical defects. The hypothesis that defects similar to those observed in mitochondrial diabetes also contribute to NIDDM is supported by studies of cybrid cells. Cybrid, or cytoplasmic hybrid, cells are constructed by fusing cells lacking nuclei (e.g., platelets) as a source of mitochondria, with cells lacking mitochondrial DNA (ρ₀ cells). The resultant cells express the mitochondrial genomes of the donor and the nuclear genome of the recipient cell. Cybrid cells constructed from individuals with NIDDM exhibited increased reactive oxygen species production and decreased ATP synthase activity compared with nondiabetic or type I diabetic individuals [Anderson et al., 1997]. Although specific mitochondrial DNA mutations that would explain this finding have not been identified, these results suggest that a defect in mtDNA is associated with some cases of NIDDM. Other investigators have shown an inverse relationship between the amount of mtDNA in peripheral blood cells and NIDDM [Lee et al., 1997]. Furthermore, these investigators reported that the decreased mtDNA content was predictive of the development of NIDDM during a 2-year observation period. This finding also supports a causal role of mtDNA alterations in the development of NIDDM. A general decrease in the levels of mtDNA could result from mutations at or near the origin of replication in the mtDNA, or could be due to an abnormality of a nuclear-encoded factor that modulates mtDNA synthesis or stability such as DNA polymerase γ or mitochondrial transcription factor A.

An additional way in which mitochondrial dysfunction may arise in NIDDM is altered expression of mitochondrial genes. Antonetti and coworkers [1995] identified several mitochondrial genes whose expression was increased in skeletal muscle of type II diabetes. In-
terestingly, these investigators also reported a 50% decrease in mtDNA copy number in the diabetics, consistent with the report by Lee and colleagues [1997]. Thus, it is unclear whether the chronic upregulation of mitochondrial mRNA expression in Antonetti’s report was secondary to the decrease in mtDNA levels or a result of the diabetic milieu or some other factor. Huang and coworkers [1997] monitored expression of NADH dehydrogenase subunit I, cytochrome oxidase subunit I, and tRNA leu in the skeletal muscle of volunteers after an euglycemic clamp. They observed decreases in the levels of NADH dehydrogenase subunit I mRNA of 54% and 14%, respectively, in NIDDM and impaired glucose tolerance compared with controls. Their study also included 12 monozygotic twin pairs who were discordant for NIDDM, but concordant for insulin resistance. No differences were observed in mRNA expression in the twin pairs. Although the authors conclude that the difference must, therefore, be acquired rather than inherited, one must keep in mind that the mitochondrial genomes of monozygotic twins may differ.

Yet another line of evidence that implicates mitochondrial dysfunction in NIDDM is the finding of increased oxidative stress in NIDDM. The production of excessive amounts of free radicals in NIDDM has been inferred from direct measurement of thiobarbituric acid reactive substances in the plasma of patients [Sato et al., 1979; Kaji et al., 1985; Uzel et al., 1987; Colllier et al., 1992; Wolff, 1993]. The levels of free radicals have also been correlated with the development of complications such as retinopathy [Uzel et al., 1987] and vascular disease [Jennings et al., 1987; Wolff, 1993]. The mechanism of free radical production has remained obscure. Possible sources of increased free radicals include the mitochondrial electron transfer chain [Baynes, 1991], nonenzymatic autooxidation of glucose or glycosylated proteins [Gillery et al., 1989; Baynes, 1991; Wolff, 1993; Kennedy and Lyons, 1997], or decreased radical scavenging [Gillery et al., 1989]. Irrespective of the cause of elevated free radicals, the potential effects include lipid peroxidation, modification of proteins, and oxidation or fragmentation of nucleic acids. Lipid peroxides are believed to contribute to the development of atherosclerosis, at least in part by their avid uptake by macrophages causing foam cell formation [Steinberg, 1987]. The accumulation of modified long-lived proteins such as collagen is thought to contribute to the development of complications that include cataracts, microangiopathy and nephropathy [Baynes, 1991; Wolff, 1993]. Oxidative damage to DNA is especially severe in the mitochondria, where protective histones are lacking and repair mechanisms are minimal (discussed above).

Although our understanding of how these processes are interrelated is improving, there is no consensus regarding whether increased free radicals are a cause or an effect of the complications of NIDDM. The role of free radicals in the development of complications has not been studied in mitochondrial diabetes. On the basis of our current knowledge, it seems that mitochondria contribute to a “vicious cycle” of free radical formation and tissue damage: increased free radicals (e.g., arising from mitochondrial electron transfer dysfunction) produce oxidative damage to mtDNA, which ultimately worsens mitochondrial dysfunction, producing greater amounts of free radicals, and so on. Thus, mitochondrial dysfunction probably contributes to the development of diabetic complications, whether by a primary defect in the mitochondria or by secondary damage to the mtDNA caused by free radicals from autoxidation or decreased radical scavenging. Additional studies will be required to define the contribution of mitochondrial dysfunction to the excess oxidative stress observed in NIDDM.

The BHE/cdb rat: An animal model of mitochondrial NIDDM

An animal model of NIDDM illustrates the importance of normal mitochondrial function in glucose homeostasis. The BHE (Bureau of Home Economics, a predecessor of the Nutrition Institute) rat strain was originated by investigators at the Food and Drug Administration, and later bred and extensively studied by Berdanier [reviewed in Berdanier, 1991; Kim and Berdanier, 1997]. Berdanier’s group described an adult onset, non-ketoacidosis-prone form of diabetes in these animals that is maternally transmitted. A defect on mitochondrial oxidative phosphorylation was ultimately characterized as a defect in ATP synthase activity. Their group identified a series of mutations in the ATP synthase 6 gene of the BHE/cdb rat compared with Sprague Dawley rat mtDNA sequenced by their group and compared with the published Wistar sequence [Mathews et al., 1995]. In collaboration with Berdanier, our laboratory confirmed their observation of decreased ATP synthase catalytic activity in BHE/cdb rats compared with Wistar rats (C. Anderson, unpublished observation). Matchinsky’s group analyzed pancreatic islets from young and elderly BHE/cdb rats and identified a secretory defect as well as loss of β-cells [Liang et al., 1994]. Thus, a mild defect in ATP synthase is associated with an abnormality of insulin secretion and the diabetic phenotype in these animals. These observations are strikingly similar to those reported for human mitochondrial diabetes.

CONCLUSION

One of the goals of this review was to summarize available data that support the idea of mitochondrial involvement in common NIDDM. The bulk of the data suggest that a defect in oxidative phosphorylation or in...
early steps of glucose metabolism, regardless of the cause of the defect, will cause impaired glucose-mediated insulin secretion. A defect in oxidative phosphorylation could result from mtDNA mutations, nuclear mutations in genes encoding mitochondrial proteins or regulatory factors, a general decrease in mtDNA levels due to decreased synthesis or increased degradation, or as a secondary effect of some other metabolic derangement. Irrespective of the cause, mitochondrial dysfunction that results in impaired ATP synthesis, impaired nucleotide translocation, or impaired NADH shuttling is predicted to cause abnormal insulin secretion.

The effects of mitochondrial dysfunction in peripheral insulin-sensitive tissues are less well understood than in the β-cell. The inconsistent occurrence of insulin resistance in the mitochondrial diseases discussed above suggests that loss of insulin sensitivity is unlikely to be a major effect of mitochondrial dysfunction. Rather, we hypothesize that the development of insulin resistance (e.g., from obesity) in a patient with underlying mitochondrial dysfunction can lead to the development of the NIDDM phenotype. A more thorough understanding of the role of mitochondria in NIDDM may present novel therapeutic and diagnostic strategies for a large proportion of the NIDDM population.

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REFERENCES


MITOCHONDRIAL DYSFUNCTION IN DIABETES MELLITUS


