Mitochondrial Diabetes: Molecular Mechanisms and Clinical Presentation

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Mitochondrial Diabetes

Molecular Mechanisms and Clinical Presentation

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Mutations in mitochondrial DNA (mtDNA) associate with various disease states. A few mtDNA mutations strongly associate with diabetes, with the most common mutation being the A3243G mutation in the mitochondrial DNA-encoded tRNA(*Leu*,*UUR*) gene. This article describes clinical characteristics of mitochondrial diabetes and its molecular diagnosis. Furthermore, it outlines recent developments in the pathophysiological and molecular mechanisms leading to a diabetic state. A gradual development of pancreatic β-cell dysfunction upon aging, rather than insulin resistance, is the main mechanism in developing glucose intolerance. Carriers of the A3243G mutation show during a hyperglycemic clamp at 10 mmol/l glucose a marked reduction in first- and second-phase insulin secretion compared with noncarriers. The molecular mechanism by which the A3243G mutation affects insulin secretion may involve an attenuation of cytosolic ADP/ATP levels leading to a resetting of the glucose sensor in the pancreatic β-cell, such as in maturity-onset diabetes of the young (MODY)-2 patients with mutations in glucokinase. Unlike in MODY2, which is a nonprogressive form of diabetes, mitochondrial diabetes does show a pronounced age-dependent deterioration of pancreatic function indicating involvement of additional processes. Furthermore, one would expect that all mtDNA mutations that affect ATP synthesis lead to diabetes. This is in contrast to clinical observations. The origin of the age-dependent deterioration of pancreatic function in carriers of the A3243G mutation and the contribution of ATP and other mitochondrion-derived factors such as reactive oxygen species to the development of diabetes is discussed. *Diabetes* 53 (Suppl. 1):S103–S109, 2004

Diabetes is a collection of diseases characterized by the presence of chronic hyperglycemia. Maintenance of normal glucose homeostasis involves the action of a glucose sensor in the pancreatic β-cell that detects an increase in blood glucose concentration and converts that into increased secretion of insulin. Increased circulating insulin concentrations suppress hepatic glucose output and stimulate glucose uptake by muscle and adipose tissue.

Pathophysiological mechanisms leading to diabetes can involve an inappropriate secretion of insulin, insulin resistance of the liver, muscle and fat, or combined defects. The risk of an individual to develop diabetes involves a complex interaction between genetic and environmental factors. Gene variants that have been identified to contribute to the major forms of diabetes, such as autoimmune type 1 diabetes and metabolic syndrome–associated type 2 diabetes, are "low penetrance" variants that modulate the susceptibility of an individual to develop diabetes or that protect against the disease (1–3).

A number of gene mutants have been identified in the past decade that represent high penetrance risk genes for diabetes. Carriers of these gene mutants have a nearly 100% chance to develop diabetes during their life span. These so-called monogenetic forms of diabetes comprise the various forms of maturity-onset diabetes of the young (MODY) (4) and mitochondrial diabetes, also called "maternally inherited diabetes and deafness" (5–7). Together, these monogenetic forms account for a few percent of the total number of diabetes cases. This review discusses our current knowledge on mitochondrial diabetes.

CLINICAL PRESENTATION OF MITOCHONDRIAL DIABETES

In clinical practice, mitochondrial diabetes generally presents itself as an unremarkable form of diabetes. The nature of the diabetes can be type 1 or type 2 in nature depending on the severity of insulinopenia. A suspicion for mitochondrial diabetes is provided by a strong familial clustering of diabetes. Although this is also seen in MODY, mitochondrial diabetes can be discriminated from MODY based on the presence of maternal transmission in conjunction with a bilateral hearing impairment in most of the carriers. Impaired hearing is detected by audiometry and reflected by a decreased perception of frequencies above 5 kHz. Final proof for the presence of mitochondrial diabetes is provided by genetic analysis. In the large majority of

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IGT, impaired glucose tolerance; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; MODY, maturity-onset diabetes of the young; mtDNA, mitochondrial DNA; OGTT, oral glucose tolerance test; ROS, reactive oxygen species; wt, wild-type.

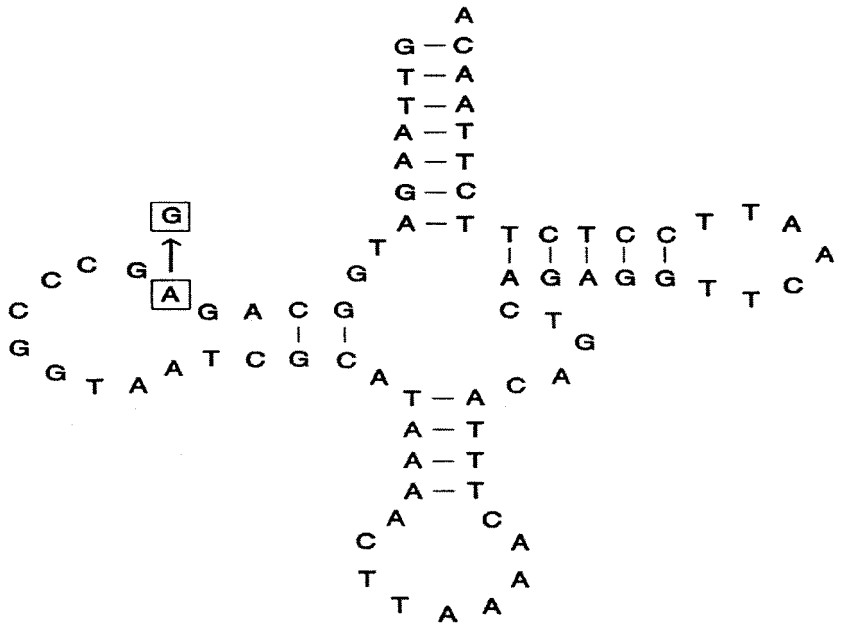

FIG. 1. Position of the diabetogenic A3243G mutation in the mitochondrial DNA-encoded tRNA(*Leu*,*UUR*) gene.

cases, mitochondrial diabetes associates with an A3243G mutation in mitochondrial DNA (mtDNA), although a range of other mutations in mtDNA have also been implicated. For a summary of these mutations, see the study by Maassen (8). The position of the A3243G mutation in the tRNA(*Leu*,*UUR*) gene is outlined in Fig. 1.

Mitochondrial diabetes associated with the A3243G mutation becomes clinically manifest at the average age of 38 years, although the range in the age of onset is quite large. Figure 2 shows a Kaplan-Meyer curve for the cumulative incidence of known diabetes/impaired glucose tolerance (IGT) in Dutch A3243G carriers. Nearly all of the carriers developed diabetes or IGT before the age of 70 years; thus, the penetrance of this mutation is nearly 100%. Diabetes can be type 1 or type 2 in nature depending on the severity of insulinopenia. Patients with a type 2–like phenotype can initially be treated by diet or sulfonylurea. Metformin is contraindicated because of the risk for lactate acidosis. Insulinopenia is progressive in nature, and most patients will require insulin treatment a couple of years after onset of diabetes. The A3243G mutation is present in heteroplasmic form (i.e., a mixture of wild-type mtDNA and mtDNA carrying the A3243G mutation [9,10]) in the patient cells.

A random screen of 1,400 blood samples sent in for HbA_{1c} determinations showed that in the Leiden region in the Netherlands, 1.3% of the samples was positive for the A3243G mutation. Another region in the Netherlands showed lower frequencies, suggesting the presence of local founders. Also, the U.K. Prospective Diabetes Study shows a low frequency among patients with a type 2 diabetes phenotype (11). Because a substantial fraction of A3243G carriers will develop a type 1–like phenotype, a selection bias may have been introduced when analyzing cohorts of diabetic patients selected for a type 2 phenotype. In Japan, the mutation seems to be present with a relatively high frequency (12). A mitochondrial haplotype analysis among Finnish individuals suggests that the A3243G mutation has occurred several times since the entry of Finnish tribes in Finland and that the number of carriers has expanded (13). Furthermore, a case of a de novo A3243G mutation in a Turkish pedigree has recently been identified by us (14). These observations suggest that the A3243G mutation is continuously generated in the human gene pool.

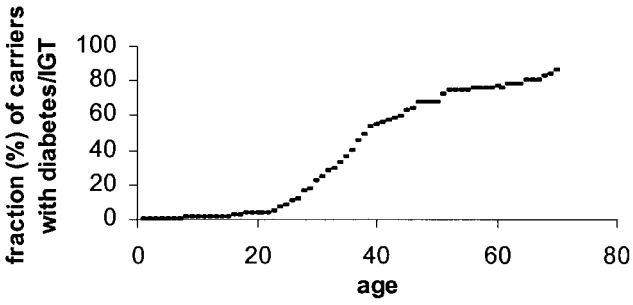

FIG. 2. Kaplan-Meyer representation of the incidence of diabetes or IGT in carriers (n = 109) of the A3243G mutation in relation to age.

Heteroplasmy levels can be quite low in leukocytes, e.g., a few percent, which hampers the detection of this mutation. Besides, heteroplasmy levels in leukocytes decline upon aging by ~0.7% per year (15). Mouth mucosa cells have on average a 1.7-fold higher heteroplasmy value than leukocytes and are the tissue of choice to detect the mutation. We observe no significant association between the degree of heteroplasmy in leukocytes and the severity of the clinical phenotype reflected by age of onset, although a trend toward a lower age of onset at high heteroplasmy values may be present. Other studies did show an association, such as in Japanese carriers (12). Thus, taken together, high heteroplasmy levels tend to predispose patients for the early onset of diabetes.

Comorbidities often reported in mitochondrial diabetes have been described previously (7). In brief, impaired hearing, reflected by a reduced perception of high tone frequencies, is highly characteristic for carriers of the A3243G mutation. Hearing impairment generally precedes the onset of clinically manifest diabetes by several years. Changes in pigmentation of the retina are also present in many carriers of the A3243G mutation.

TABLE 1
Characteristics of the study cohort examined by a hyperglycemic clamp

	n	BMI (kg/m^2)	Fasting glucose (mmol/l)	HbA_{1c} (%)	First-phase insulin (pmol/l)*	Second-phase insulin (pmol/l)†
Noncarriers	90	28.4	6.56	5.7	694	312
A3243G carriers	2	24.7	6.35	5.9	390	141

*First-phase integrated insulin concentrations at 2.5, 5, 7, and 10 min after application of 10 mmol/l hyperglycemia. †Second-phase average insulin concentration in the 120- to 180-min range, minus basal.

Other comorbidities that seem to be present more frequent than just by chance are gastrointestinal abnormalities, cardiomyopathy, and renal dysfunction with resemblance in its clinical course to the Alport syndrome (12,16,17).

The A3243G mutation was originally detected in patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS syndrome) (18). In our Dutch group of carriers ($n = 120$), we only see two cases with clinically manifest signs of MELAS. It seems that in Japanese individuals, MELAS is more commonly associated with the A3243G mutation, although the nature of selection of patients may introduce an ascertainment bias.

PATHOPHYSIOLOGICAL MECHANISMS LEADING TO DIABETES

The A3243G mutation is present in all tissues, although heteroplasmy levels tend to be high in tissues with a low mitogenic activity, such as muscle. It was originally considered that the main defect leading to diabetes is an altered glucose metabolism of muscle. Additional studies did not identify insulin resistance as a common factor in most carriers of the A3243G mutation, although insulin resistance has been reported in some carriers. Furthermore, energy metabolism in muscle, as reflected by ATP/ADP and phosphocreatine levels under conditions of rest and exercise, is not strongly deregulated (E.v.E., J.A.M., H.H.P.J.L., unpublished data).

Hepatic glucose production may be another factor that becomes deregulated by the A3243G mutation. A mitochondrial dysfunction in muscle is expected to lead to a higher lactate flux to the liver, fueling gluconeogenesis. At this time, no data are available on hepatic glucose production and its suppression by insulin in carriers of the A3243G mutation.

We have detected an impaired pancreatic insulin secretion in response to glucose stimulation in carriers of the A3243G mutation. We performed a hyperglycemic clamp at 10 mmol/l glucose on a group of 92 individuals with IGT. These individuals participated in a population-based study on glucose intolerance in the general population of Hoorn, the Netherlands, and were selected as having IGT based on two consecutive oral glucose tolerance tests (OGTTs). Two individuals were found to be carriers of the A3243G mutation. The characteristics of this group are given in Table 1. These two carriers showed significantly lower insulin secretion than the noncarriers (Fig. 3). When insulin sensitivity was estimated from the ratio between the glucose disposal rate at 10 mmol/l glucose and the ambient insulin levels, a higher insulin sensitivity was noted in both A3243G carriers compared with noncarriers with IGT (0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1} \cdot l^{-1}$ in noncarriers vs. 0.27 in carriers, $P < 0.01$). We also examined 25 nondiabetic carriers of the A3243G mutation and 25 age-, sex-, and BMI-matched noncarriers by OGTT. No significant differences in glucose, insulin, C-peptide, and glucagon concentrations were seen after the 75-g glucose load. These findings indicate the absence of detectable consequences of the presence of the A3243G mutation before development of glucose intolerance.

WHY IS THE A3243G MUTATION STRONGLY DIABETOGENIC COMPARED WITH OTHER MITOCHONDRIAL DNA MUTATIONS?

A large number of mtDNA mutations have been identified in disease states. Some mtDNA mutations have as a hallmark an impaired muscular function, such as in the case of myoclonic epilepsy and ragged red fibers, which associate with mutations at position 8344 in the tRNA(Lys) gene. Other mutations predominantly affect the functionality of the optic nerve, such as mutations at position 11778 in mtDNA, which associate with Lebers hereditary optic neuropathy (19). Thus, individual mutations tend to associate with distinct syndromes. It has been suggested that the disease state results from an attenuated generation of ATP from ADP by mutant mitochondria. To explain variations in clinical presentations, it has been suggested that the degree of heteroplasmy and its distribution over particular tissues may determine the nature of the clinical phenotype. Heteroplasmy values show large variations between individual tissues with a tendency toward high heteroplasmy values in nondividing tissue compared with

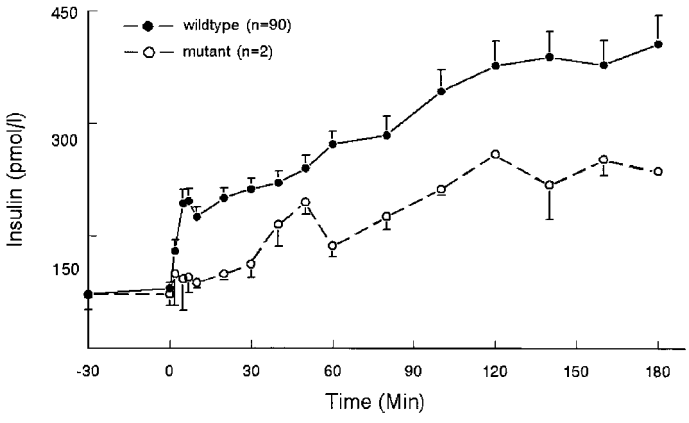

FIG. 3. Insulin secretion during a hyperglycemic clamp in A3243G carriers compared with noncarriers. Individuals with IGT ($n = 92$) were randomly selected, and insulin secretion was determined by applying 10 mmol/l glucose at the 0-min time point. Insulin secretion was followed during 180 min. Two individuals were carriers of the A3243G mutation.

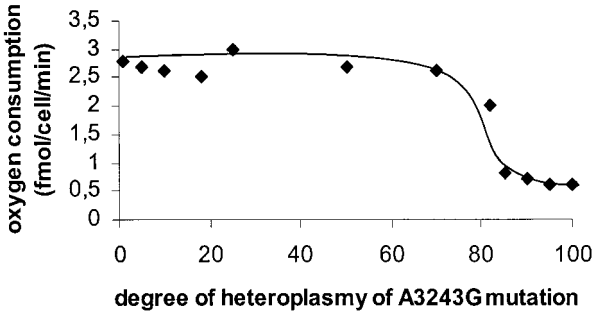

FIG. 4. The rate of oxygen consumption in relation to heteroplasmy levels for the A3243G mutation. Human osteosarcoma cells were repopulated with donor mitochondria originating from fibroblasts from patients carrying the A3243G mutation in the heteroplasmic state. Individual clones were isolated, and heteroplasmy levels and the rate of oxygen consumption were determined. Oxygen consumption for each clone is represented in relation to the degree of heteroplasmy. A 50% decline in the rate of oxygen consumption occurs at ~85% heteroplasmy.

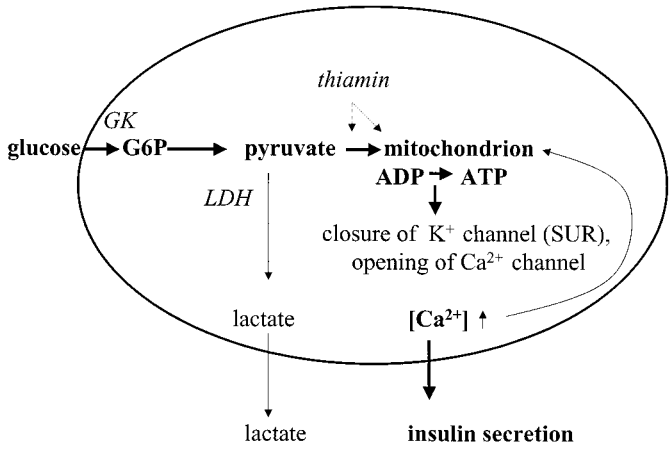

FIG. 5. Schematic overview of the steps linking variations in glucose concentration to variations in insulin secretion. The efficiency by which variations in glucose concentrations are coupled with the magnitude of the change in ADP/ATP ratio is a main determinant for insulin secretion. Steps that modulate the efficiency are as follows: the activity of glucokinase (GK); the activity of lactate dehydrogenase (LDH), which is low in functional β-cells (thin arrows); and the thiamin status of the cell. Thiamin is a cofactor for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, enzymes that determine the flux of glucose through glycolysis and the Krebs cycle. The activity of the Krebs cycle is also upregulated by the uptake of Ca^{2+} by mitochondria after the ATP/ADP-induced closure of the K channel and subsequent opening of the voltage-gated Ca channel. G6P, glucose-6-phosphate.

rapidly dividing cells. Heteroplasmy values even show a high degree of variability at the individual cell level (20). Thus, variations in the distribution of heteroplasmy levels are plausible factors in setting the clinical phenotype, although they do not fully explain the presence of distinct clinical entities with individual mutations.

The presence of the A3243G mutations in hybrid cell lines (i.e., cells repopulated with donor-derived wild-type [wt] or mutant mitochondria) above a certain threshold in heteroplasmy values leads to a reduction in oxygen consumption and oxidative phosphorylation. Figure 4 illustrates this phenomenon. This threshold is at 80–90% mutant mitochondria—values that are generally not seen in patients with the A3243G mutation (21). The mutation itself leads to dimerization of the mutant tRNA molecule and impaired aminoacylation (22). The precise consequences for biochemical processes within the mitochondrion is a matter of debate but seems to involve an unbalance in the amount of mitochondrial DNA-encoded proteins. Furthermore, high heteroplasmy levels lead to attenuated oxygen consumption, indicating functional impairment of the respiratory chain (22–26).

The deleterious consequences of mtDNA mutations are often considered from the viewpoint of impaired ATP production, although we feel that effects on additional cellular processes in which mitochondria are involved should be taken into account too. In pancreatic β-cells, the ATP/ADP ratio determines the opening probability of the K_{ATP} channel involved in insulin secretion, and changes in this ratio as a result of mitochondrial dysfunction will certainly affect the setting of the glucose-induced insulin secretion response, as illustrated in Fig. 5. In agreement with this concept are the observations that pancreatic β-cell lines in which the mtDNA content is strongly reduced by culture in the presence of ethidium bromide do show a loss of glucose-induced insulin secretion, although the closure of the K channel by sulfonylurea-related pharmaceuticals still stimulates insulin secretion (27,28). Experiments in which mtDNA copy number is reduced in pancreatic β-cells by tissue-specific knockout of the nuclear-encoded gene for mitochondrial transcription factor A in pancreatic β-cells support the concept of limiting mitochondrial function in insulin secretion (29). Several observations are more difficult to reconcile with this straightforward concept. Mitochondrial diabetes only develops upon aging. In the case of MODY2, in which a defect in the first step of glycolysis results in attenuated ATP generation in response to glucose, hyperglycemia is already manifest early after birth (30). Mitochondrial diabetes, however, has on average an age of onset between 35 and 40 years. Thus, it seems that in the case of mitochondrial diabetes, aging-related processes contribute to the gradual deterioration of β-cell function.

The diabetogenic nature of the A3243G mutation has also been explained from the viewpoint that this mutation may be present at high heteroplasmy, specifically in pancreatic β-cells. In those few cases where postmortem heteroplasmy levels in individual tissues were determined, no convincing relation emerged between heteroplasmy distribution and the clinical presentation (30a,31).

We propose that additional signaling molecules next to ATP/ADP contribute to the pathogenesis of mtDNA-related diseases. Mitochondria contribute to the regulation of multiple compounds in the cytosol; many of them are involved in cellular regulation, such as Ca, glutamate, cytochrome C, and lactate (32–35). Furthermore, mitochondria are a main source of radicals. Distinct mutations may differentially affect concentrations of these signaling molecules, thereby leading to different consequences for cellular properties and the disease state.

To understand the strong diabetogenic character of the A3243G mutation compared with other mtDNA mutations, we hypothesized that the underlying factor involves a deregulation of the complex interaction between mitochondrial function and nuclear gene expression. To understand the nature of this crosstalk between mitochondrial and nuclear genomes, we analyzed gene expression patterns in human cells with two different types of mitochondrial dysfunction. One type of dysfunction is reflected by

the presence of A3243G mutant mitochondria. Another type of mitochondrial dysfunction is reflected by the presence of a strongly reduced number of mitochondrial DNA copies in each cell. The effect of these conditions on gene expression patterns was analyzed by Affymetrix oligonucleotide microarrays comprising ~33,000 genes. For comparison, cells expressing normal numbers of WT mitochondria were included too. When comparing cells with reduced mtDNA copy number and cells with the A3243G mutant, some gene clusters were similarly affected by the presence of the A3243G mutation and reduced copy number, whereas other gene clusters showed differential deregulation. In particular, expression levels of 25 ribosomal protein genes were strongly reduced only in A3243G mutant cells compared with cells with normal copy numbers of wt mitochondria.

Thus, the consequences for cellular gene expression by the presence of the A3243G mutation compared with a reduction in mtDNA copy number are quite distinct and cannot be explained by a mere reduction in the rate of ATP synthesis. The diabetogenic nature of the A3243G mutation compared with other mtDNA mutations may be linked to this phenomenon and is currently being explored.

Studies on pancreatic β-cell lines with reduced mtDNA copy numbers and the observation that mutations in glucokinase (MODY2) and cellular thiamin deficiency, as seen in the thiamine-responsive megaloblastic anemia with diabetes and deafness syndrome, are also diabetogenic have been explained on the basis of the critical role of the ATP/ADP ratio in the process of insulin secretion (27,28,30,36). A more recent study on the pancreatic β-cell line βHC9 showed that mtDNA depletion did not significantly affect basal cytosolic ATP concentrations, although glucose-induced insulin release was blocked. This observation also suggests that mitochondrial function itself contributes to the process of insulin secretion (37).

WHAT CAUSES THE AGE-DEPENDENT DETERIORATION OF β-CELL FUNCTION IN MITOCHONDRIAL DIABETES?

Nondiabetic carriers of the A3243G mutation initially have a normal insulin/C-peptide secretion during an OGTT. Upon aging, a progressive deterioration of glucose homeostasis occurs. Insulin resistance does not seem to be a major causative factor. Rather, a progressive loss of insulin secretion in response to glucose seems to be the major factor. This situation is in contrast to carriers of glucokinase mutations who suffer from a nonprogressive form of diabetes, which is already manifest early after birth. Because no evidence exists that heteroplasmy levels for the A3243G mutation increase upon aging, other factors must be involved in the time-dependent deterioration of β-cell function. At this moment, these factors remain to be identified, although an enhanced age-related β-cell death seems a plausible factor.

Mitochondria are main producers of reactive oxygen species (ROS) inside cells (Fig. 6). The amount of ROS contributes to apoptosis and probably also to the differentiation state of pancreatic β-cells. Pancreatic β-cells have a poor regeneration capacity when cells are lost. Thus, under conditions of enhanced loss of β-cells, either by apoptosis or dedifferentiation, a situation will emerge in which insufficient replacement takes place of lost β-cells.

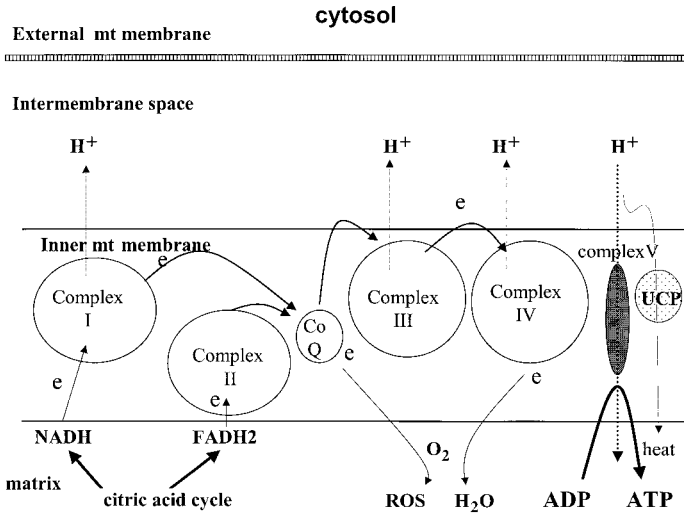

FIG. 6. Overview of the mitochondrial respiratory chain and the steps that contribute to formation of ROS. Electrons (e) originate from NADH and FADH2 that are predominantly generated via the Krebs (citric acid) cycle. The transfer of electrons through the various complexes of the respiratory chain is coupled with the pumping of protons from the mitochondrial matrix to the intermembrane space. The resulting proton gradient drives the ATP synthesis at complex V (ATP synthase). Part of the protons may leak out through uncoupling proteins (UCP) without generating ATP. Normally, electrons transported through the respiratory chain are transferred at or near complex IV to oxygen, yielding in the end H_2O. Electrons present at coenzyme Q (Co Q) in the respiratory chain may be transferred to oxygen yielding ROS. A high proton gradient may slow down the rate of electron transport through the chain, thereby enhancing ROS production.

In the end, this will result in inadequate insulin secretion to maintain correct glucose homeostasis. Enhanced ROS production may facilitate this process. Theoretically, the amount of mitochondrial ROS production depends on a number of factors. A high mitochondrial membrane potential seems to favor generation of ROS. This situation occurs under conditions of low levels of uncoupling proteins, as seen in mice lacking uncoupling protein 3 (38). This observation implies that a high rate of NADH/$FADH_2$ formation at a limiting supply of ADP also enhances ROS production. Conditions favoring this situation are high glucose levels in combination with elevated intracellular Ca—a situation expected to be present in pancreatic β-cells under hyperglycemic conditions. Pancreatic β-cells have low lactate dehydrogenase activity (39). Thus, most of the glucose that is taken up by the cell will contribute to the generation of mitochondrial membrane potential. Simultaneous elevation of cytosolic Ca leads to an influx of Ca into mitochondria, where Ca acts as a cofactor for rate-limiting enzymes of the citric acid cycle (40). Thus, more NADH/$FADH_2$ is produced, which is expected to lead to an increased buildup of membrane potential over the mitochondrial inner membrane. Normally, ADP serves to reduce the membrane potential by formation of ATP. Under conditions of hyperglycemia, ADP regeneration may reach a maximum, which depletes mitochondria of ADP, and the resulting high membrane potential is expected to result in enhanced ROS production. This may contribute to dedifferentiation of pancreatic β-cells and a loss of responsiveness toward glucose (41). Interestingly, the availability of ADP is also determined by the activity of the adenine nucleotide translocator, which mediates ADP-

ATP exchange between mitochondrial matrix and cytosol. High concentrations of fatty acid-CoA esters, a situation that is expected to exist in obese individuals, seems to reduce the activity of the adenine nucleotide translocator (42), thereby favoring the formation of ROS. These mechanisms may explain the well-known observation of gluco- and lipotoxicity for β-cells.

Another condition favoring ROS production is a reduction in mitochondrial copy number per cell. Each mitochondrion exhibits an increased workload under these conditions, leading to high membrane potential and ROS formation. In vivo, reduced mtDNA copy number can be induced by inhibition of mtDNA polymerase by dideoxynucleotide analogs. These drugs are used for antiretroviral therapy. When applied in vivo, they enhance the risk for insulinopenic diabetes and pancreatitis (43,44). These observations indicate that maintenance of an appropriate mtDNA copy number is critical for appropriate in vivo insulin secretion.

Factors that reduce ROS formation are elevated expression levels of uncoupling proteins (45). Although these uncouplers reduce the magnitude of the glucose-induced insulin secretion response itself, making the β-cell less efficient, they may protect the β-cell against elevated ROS production.

It is unknown whether the presence of low levels of A3243G mutation induces a state of enhanced ROS production, thereby contributing to premature aging of pancreatic β-cells.

In summary, most data support the concept that a correct mitochondrial function is critical for maintenance of an adequate glucose-induced insulin secretion. In case of the A3243G mutation and other more rare mtDNA mutations, the overall capacity of mitochondrial function becomes rate-limiting to sustain adequate levels of insulin secretion. In addition, an accelerated deterioration of β-cell function is seen. What precisely causes this premature aging and whether it also plays a role in β-cell dysfunction in common type 2 diabetes remains to be established.

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