Mitochondrial diabetes and its lessons for common Type 2 diabetes


*Department of Molecular Cell Biology, Leiden University Medical Centre, 2300 RC Leiden, The Netherlands, and ‡Department of Endocrinology and Metabolic Diseases, Leiden University Medical Centre, 2300 RC Leiden, The Netherlands

Abstract

Multiple pathogenic pathways are able to deregulate glucose homoeostasis leading to diabetes. The 3243A>G mutation in the mtDNA (mitochondrial DNA)-encoded tRNALeu,UUR gene was found by us to be associated with a particular diabetic subtype, designated MIDD (maternally inherited diabetes and deafness). This mutation causes an imbalance in the mitochondrion between proteins encoded by the nuclear and mitochondrial genomes, resulting in a gradual deterioration of glucose homoeostasis during life. Remarkably, carriers of the 3243A>G mutation are generally not obese. The mutation also results in enhanced radical production by mitochondria. We propose that this mutation leads to the development of diabetes due to an inappropriate storage of triacylglycerols within adipocytes. The result is a fatty acid-induced deterioration of pancreatic β-cell function. In combination with an enhanced radical production in the β-cell due to the mutation, this leads to an age-dependent, accelerated decline in insulin production.

Introduction

Diabetes is a disease characterized by persistent hyperglycaemia. Several subtypes of the disease exist with multiple underlying pathogenic pathways. The most common form of diabetes is Type 2 (non-insulin-dependent) diabetes in which obesity-associated insulin resistance of the target tissues for insulin is an early marker of the disease process. If the resulting increased demand for insulin cannot be met by an increased production of insulin by the pancreas, clinically manifest diabetes develops. Risk factors to development of the disease are a genetic predisposition and a westernized life style with obesity and little physical exercise. Alterations in triacylglycerol metabolism leading to the accumulation of visceral fat are early steps in the disease process which precede the development of glucose intolerance [1]. Fatty acids in the circulation induce insulin resistance in multiple cells/tissues and, furthermore, fatty acids are toxic to pancreatic β-cells [2]. Based on these experimental observations, an increased exposure of the body cells to fatty acids has been suggested as a major pathogenic factor involved in the development of Type 2 diabetes.

Several mutations in mtDNA (mitochondrial DNA) have been found to be associated with enhanced risk for developing diabetes [3]. Most of them are rare. One particular mutation, the 3243A>G mutation in the mtDNA-encoded tRNALeu,UUR gene is associated with the MIDD (maternally inherited diabetes and deafness) syndrome and accounts for 0.2–2% of the diabetic cases [4–7]. Highest frequencies are found in Japan. The penetrance of this mutation is high, as >85% of the carriers will develop diabetes during life [8]. Furthermore, a genetic variant in the nuclear-encoded LARS2 (leucyl-tRNA synthetase 2) gene that encodes the mitochondrial leucyl-tRNA synthetase also modulates the risk to develop diabetes [9], indicating that a decline in mitochondrial protein synthesis involving the incorporation of leucine is critical for proper glucose homoeostasis. Remarkably, the 3243A>G mutation is also associated with the MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) syndrome [10]. The origin of the variability in clinical expression of the 3243A>G mutation is unknown.

We will discuss the clinical phenotype of MIDD in relation to underlying biochemical mechanisms by which the 3243A>G mutation leads to diabetes. Furthermore, we will discuss to what extent a mitochondrial dysfunction is also involved in the pathogenesis of common Type 2 diabetes.

Results and discussion

Clinical phenotype of MIDD

Carriers of the 3243A>G mtDNA mutation develop diabetes on the average at 35–40 years of age. In general, the...
Figure 1 | Outline of the biochemical pathway involved in activating the process of glucose-induced insulin secretion in pancreatic $\beta$-cells

The biochemical steps that result in deregulated insulin secretion in the neonatal state in case of an altered function of those steps are indicated in boldface. GK, glucokinase; Glut2, glucose transporter 2; G6P, glucose-6-phosphate; PDH, pyruvate dehydrogenase.

Patients are not obese [5,6,8]. The clinical data suggest that carriers of the 3243A $>$ G mutation (normoglycaemic, impaired glucose tolerance and diabetic individuals) do not exhibit insulin resistance. Rather they exhibit reduced glucose-induced insulin secretion when glucose intolerance develops [11]. In normoglycaemic carriers of the 3243A $>$ G mutation, no abnormalities in circulating glucose and insulin concentrations are observed during oral glucose tolerance tests [8]. These findings suggest that early in life, the 3243A $>$ G mutation is not phenotypically expressed when glucose homeostasis is considered and that somewhere during midlife $\beta$-cell function declines. The carriers of the mutation also exhibit an accelerated loss of perception of high tone frequencies, a process that resembles the normal age-related decline in perception of high tone frequencies. Thus an accelerated aging of the auditory apparatus seems to be associated with the 3243A $>$ G mutation. This may also point to a premature aging of other organs, such as pancreatic $\beta$-cells.

Pathobiochemistry of the 3243A $>$ G mutation

Cells contain multiple mitochondria and each mitochondrion carries a few mtDNA molecules. Pathogenic mutations in mtDNA are generally present in only a fraction of the mtDNA population within a cell, a situation called heteroplasmy [12].

In cybrid cell lines in which the fraction of mutant 3243A $>$ G mitochondria exceeds approx. 75%, respiration is diminished, and in cybrids with 100% mutant mitochondria, respiration is only 10% of wild-type mitochondria [8]. Thus the mutant mitochondria have a strongly diminished activity to perform respiration and to synthesize ATP. The results also show that an excess of mitochondrial capacity exists in these cells when respiration is considered, allowing a major fraction of the mitochondrial population to be non-functioning before respiration declines.

The mechanism leading to the mitochondrial dysfunction by the 3243A $>$ G mutation is a reduction in protein synthesis of mtDNA-encoded proteins, leading to an imbalance within the mitochondrion between mitochondrial proteins encoded by the nuclear and mitochondrial genomes [13,14].

Individuals with the 3243A $>$ G mutation develop diabetes as a result of a decline in the secretion of insulin. This may result from a diminished number of functional $\beta$-cells or from a resetting of the glucose sensor. A decline in the number of functional $\beta$-cells is difficult to determine in the human situation and only a few data are available from post-mortem studies. These suggest that 3243A $>$ G carriers may have a diminished number of $\beta$-cells compared with the number of $\alpha$-cells in the endocrine pancreas [15,16]. A change in the setting of the glucose sensor as a result of the 3243A $>$ G mutation is also a plausible explanation for the decline in glucose-induced insulin secretion. This sensor involves a glucose-induced increase in the cytosolic ATP/ADP ratio within the $\beta$-cell as a result of an increased metabolic flux through glycolysis and the tricarboxylic acid cycle (Figure 1). A mitochondrial dysfunction could decrease the glucose-induced increase in the ATP/ADP ratio and, thereby, the
amount of secreted insulin. This could result in an inappropriate secretion of insulin to meet the demand and thereby leading to the diabetic phenotype. Although this mechanism seems plausible, there are several observations that argue against this pathogenic mechanism.

Mutations in the glucokinase gene (Figure 1) that controls the carbohydrate flux to mitochondria and, thereby, the ATP/ADP ratio and the opening state of the K^+ channel lead to a permanent state of glucose intolerance. Both hyperglycaemia with insufficient insulin secretion as in MODY2 (maturity-onset diabetes of the young 2) [17] and hypoglycaemia with neonatal hyperinsulinaemia are seen, dependent on the nature of the mutation [18]. Hypo- or hyperglycaemia is already manifest early after birth. Also an intracellular deficiency in thiamine which decreases the glycolytic flux at the step of pyruvate dehydrogenase leads to secretion of insufficient amounts of insulin early in life [19]. Furthermore, mutations in genes encoding proteins of the ATP/ADP-controlled K-channel in the β-cell result in neonatal hypo- or hyper-insulinaemia [20]. This situation is in contrast with the situation seen in case of the 3243A > G mutation where it takes on the average 35–40 years before glucose intolerance develops [8]. This is a strong argument against a simple resetting of the pancreatic glucose sensor as a result of the 3243A > G mutation and suggests a contribution of age-related processes to the gradual impairment of β-cell function. Further arguments against a decline in ATP production as major pathogenic factor comes from the following observations. In cybrid cell lines, respiratory chain function and ATP synthesis are affected only when more than 70% of mtDNA carries the mutation. In diabetic patients, the 3243A > G mutation is often present in heteroplasmy levels of 2–20% in leucocytes. Although it is difficult to extrapolate these heteroplasmy values in leucocytes to precise values for other tissues, available post-mortem data suggest heteroplasmy values far below 70% in pancreatic tissue [15,16]. Yet, these individuals develop diabetes. Thus it seems that the mutation acts in a dominant way. This implies that it is unlikely that merely the diminished number of fully functional mitochondria, able to synthesize ATP, is the causative factor leading to diabetes. An additional argument against a decreased maximal capacity to synthesize ATP as a major pathogenic factor comes from the observation that distinct mutations in mtDNA, which all affect ATP synthesis, cause distinct syndromes, often diabetes not being part of the syndrome. Thus additional pathogenic pathways that are linked to the 3243A > G mutation, other than a diminished ATP synthesis, must contribute significantly to the disease process.

Another plausible mechanism to explain the decline in glucose-induced insulin secretion involves a decreased capacity of the β-cell carrying the 3243A > G mutation to synthesize insulin. This situation may result from activation of AMPK (AMP-activated protein kinase) due to a reduced synthesis of ATP. Activation of AMPK decreases cytosolic ribosomal protein synthesis through the mTOR (mammalian target of rapamycin)–S6 kinase pathway [21]. In this model, as discussed above, one would expect that all other pathogenic mutations in mtDNA that affect ATP synthesis would yield a diabetic phenotype which, apparently, is not the case. Furthermore, also this model is difficult to reconcile with the age-dependent decline in β-cell function.

Electron microscopy pictures from fibroblasts and cybrid cell lines expressing mutant mitochondria show the presence of cytosolic triacylglycerol droplets, indicating a diminished ability of the 3243A > G mutant mitochondria to perform β-oxidation of fatty acids (Figure 2). In addition, studies by Maechler and co-workers [22] have shown that, in cybrid cell lines, the presence of mitochondria with the 3243A > G mutation results in an enhanced oxidative stress which is expected to result in the random introduction of somatic point mutations in mtDNA. This leads to an accelerated aging of the β-cell and enhanced apoptosis. Accumulation of point mutations in mtDNA by introducing proof-reading-defective mtDNA polymerase in transgenic mice has been shown to result in premature aging of these animals [23,24]. The increased mutational load in mtDNA resulting from the
Figure 3 | Pathogenic mechanisms leading to β-cell dysfunction in obesity-associated Type 2 diabetes and MIDD
For further details, see text. fa, fatty acid.

3243A > G mutation may be further enhanced by enhanced lipotoxicity due to a diminished capacity of adipocytes to adequately store triacylglycerols in carriers of the 3243A > G mutation. Together, these processes explain the age-dependent, accelerated decline in β-cell function and the observation of a decline in the ratio of β-cells/α-cells in the pancreas of 3243A > G carriers [15].

Does a mitochondrial dysfunction contribute to common Type 2 diabetes?
A lower expression of genes encoding the mitochondrial machinery has been observed in muscle biopsies from individuals with Type 2 diabetes and their relatives [25,26] and this has triggered again the search for a contribution of a change in mitochondrial function to the pathogenesis of common Type 2 diabetes.

The hallmark of Type 2 diabetes is insulin resistance, which is absent from MIDD, and an age-dependent decline in β-cell function, which is also seen in MIDD. The pathophysiology of Type 2 diabetes seems to involve an inappropriate storage of triacylglycerols in peripheral adipose tissue, leading to hepatic steatosis and fatty acid-induced toxicity of the β-cell. Fatty acids and cytokines from inflamed adipose tissue contribute to the development of insulin resistance in Type 2 diabetes [27]. The observed diminished expression of genes encoding mitochondrial proteins in muscle from Type 2 diabetics and its first-degree relatives suggests that a diminished mitochondrial function is involved in the development of insulin resistance and/or diabetes [25,26]. On the other hand, hyperglycaemia is able to down-regulate expression of respiratory-chain-encoding genes [28] making it difficult to distinguish between cause and consequence of the observed effects. Furthermore, as individuals with the 3243A > G mutation generally do not exhibit whole body insulin resistance, this argues against a direct involvement of a mitochondrial dysfunction in the development of muscle insulin resistance. Recently, it was shown that the differentiation of 3T3L1 cells into adipocytes is associated with a marked increase in mitochondrial numbers [29]. This is remarkable as the main function of white adipose tissue is to store triacylglycerols without an apparent need for a high mitochondrial content. Furthermore, in animals with obesity-related diabetes, mitochondrial function is notably decreased in adipose tissue [30]. Insulin sensitizers from the TZD (thiazolidinedione) family improve mitochondrial function in adipocytes by increasing mitochondrial content [29,31]. As mitochondria in cells scavenge fatty acids by β-oxidation, the presence of large amounts of functional mitochondria within adipocytes may be a safeguard to prevent inadvertent release of fatty acids by adipocytes under conditions of inappropriate suppression of lipolysis, e.g. occurring in the insulin-resistant state. Thereby, an increased number of functional mitochondria in adipocytes can improve whole-body insulin sensitivity and diminish lipotoxicity to the β-cell. Thus, in obesity-associated Type 2 diabetes, the following sequence of events may occur: insulin-resistant, enlarged adipocytes release elevated amounts of fatty acids because of incomplete suppression of lipolysis, together with
inflammatory cytokines. An overload of the β-oxidation machinery in mitochondria within the adipocyte occurs, leading to a further deterioration of mitochondrial function and a subsequent further decline in removing fatty acids. As a result, systemic overload with fatty acids occurs, especially under conditions of little physical exercise, as the active muscle represents normally a major sink for fatty acids. As a result, an accelerated deterioration of β-cell function occurs due to fatty-acid-induced mitochondrial toxicity. The mechanism by which TZDs improve Type 2 diabetes may, at least in part, be mediated by decreasing the exposure of tissues to fatty acids by improving the mitochondrial function in adipocytes.

Most carriers of the 3243A > G mutation tend not to be obese and this situation suggest that a metabolic change has also occurred in their adipocytes so that they are less capable of storing triacylglycerols. This situation bears some resemblance to the partial lipodystrophy which is induced by HAART (highly active antiretroviral therapy) and which is associated with insulin resistance and increased risk for Type 2 diabetes [32]. HAART results in partial inhibition of mtDNA polymerase and a decreased mtDNA copy number in cells [32]. Under those conditions, it can be expected that β-oxidation of fatty acids is also diminished, resulting in enhanced fatty acid release by adipocytes and increased lipotoxicity to the β-cell.

In conclusion, these considerations suggest that during the pathogenesis of common Type 2 diabetes, a fatty acid-induced mitochondrial dysfunction in adipocytes initiates a process of overflow in other tissues, with excess of fatty acids. In the pancreas, this process leads to lipotoxicity, damage to mtDNA and a premature aging of the β-cell. In carriers of the 3243A > G mutation, pancreatic β-cells undergo accelerated aging due to enhanced free radical production as a result of the 3243A > G mutation itself. In addition, due to the poorly developed adipocytes as a result of the 3243A > G mutation, a continuously elevated exposure of tissues to fatty acids exists, further enhancing the age-dependent decline in insulin secretion by the β-cell. Figure 3 summarizes this concept. Further studies are needed to corroborate this concept and to identify why carriers of the 3243A > G mutation generally are not obese and insulin-resistant.

References


Received 22 June 2006