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Mitochondrial endocrinology

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Introduction

It can be argued that the first mitochondrial disease ever described was initially evaluated in an endocrine clinic. In 1962, Rolf Luft reported the case of a young woman who suffered from euthyroid hypermetabolism.[1] Biochemical and ultrastructural studies were suggestive of a defect in the coupling efficiency of this patient's mitochondria. While the molecular etiology of 'Luft disease' is still not known today, this initial case report sparked the creation of 'mitochondrial medicine.'

There appears to be an ever-expanding role for the mitochondrion in both rare and common human diseases. Key cellular processes, such as oxidative phosphorylation, apoptosis, steroid and lipid biosynthesis, and intermediary metabolism, are situated in this organelle, linking it to virtually all organ systems. Evolutionary and genetic studies suggest that environmental factors, such as climate and food supply, may have shaped the pattern of mitochondrial DNA haplotypes across the world, contributing to the spectrum of common human diseases observed today.[2] Although mitochondrial dysfunction, resulting either from mutations in mtDNA or in nuclear DNA, has traditionally been associated with encephalomyopathy, it is now clear that virtually all organ systems can be affected.

Endocrine dysfunction appears to be a very common manifestation of mitochondrial disease. Chinnery and Turnbull have gone so far as to suggest that diabetes may be the most common mtDNA disease phenotype.[3] A recent survey of patients with documented respiratory chain disease reported that following the nervous system, the endocrine system is most frequently affected in these patients. In fact, nearly 50% of these patients had some form of endocrine dysfunction.[4] Clinicians caring for patients with mitochondrial disease need to become increasingly aware of its endocrine manifestations, since most of the disorders are treatable. When endocrine organ involvement is suspected, referral to an endocrinologist may help in guiding the diagnosis and treatment. Improvement of the endocrine disorder may also have benefit to other affected systems.

Here we review the various endocrine disorders that can arise in mitochondrial diseases, with a special focus on diabetes.

Mitochondria and diabetes

Diabetes represents a collection of diseases characterized by chronic hyperglycemia, and is one of the leading causes of cardiovascular disease, stroke, limb loss, blindness, and renal failure.[5] Diabetes mellitus is characterized and defined by hyperglycemia. Current American Diabetes Association criteria for the diagnosis include either a fasting blood glucose > 126 mg/dL or a random glucose > 200 mg/dL on two occasions. An oral

glucose tolerance test can also be used to establish the diagnosis with a 2-hour glucose value greater than 200 mg/dL.[6] Maintenance of normal glucose homeostasis requires the action of a glucose sensor in the pancreatic β-cell that detects increases in circulating glucose and converts this signal into increased insulin secretion. Increased insulin then suppresses glucose output from the liver and promotes glucose uptake in peripheral tissues such as skeletal muscle and adipose tissue.

Diabetes can result from an impaired secretion of insulin by the β-cell, as well as by a loss of its action (termed insulin resistance) in peripheral tissues, such as skeletal muscle, fat and liver. It has long been known that increased insulin resistance can eventually lead to decline in function of the pancreatic β-cell. Likewise, an impaired secretion of insulin can result in hyperglycemia, eventually leading to peripheral insulin resistance. This dynamic relationship often makes it difficult to pinpoint the primary event leading to diabetes. Diabetes is the prototypical complex disease, as many genes (individually and in combination) and environmental factors (e.g., diet, exercise, drugs) can contribute to its heritability, age of onset, and severity.[7]

Recent studies have suggested that the mitochondrion may lie at the heart of all forms of non-immune diabetes.[8–12] The hypothesis that either inherited or acquired mitochondrial dysfunction may underlie all forms of diabetes may help unify a number of observations gleaned through genetics, clinical medicine, epidemiology, and pharmacology. This idea would also be consistent with the thrifty gene hypothesis,[13] which states that genotypes which were selected for during times of food or water scarcity may be detrimental during times of food surplus. In this section, we review the clinical features of mitochondrial diabetes, due to mutations in mtDNA, as well as some of evidence supporting the notion that this organelle may underlie all forms of non-immune diabetes.

Mitochondrial diabetes due to variation in mtDNA

By general consensus mtDNA mutations cause approximately 1.5% of cases of diabetes in Europe, and perhaps as much as 5% of diabetes in East Asia, particularly in Japan.[14]

Clinically, mitochondrial diabetes typically presents as an unremarkable form of diabetes, sometime between 22 and 35 years of age, between the peak ages of onset for type 1 diabetes and type 2 diabetes or maturity-onset diabetes of the young (MODY).[15] The disorder is maternally inherited and can be characterized by a defect in insulin secretion or occasionally by insulin resistance. Interestingly, the majority of these patients are thin (BMI < 25 kg/m^2). Although most eventually require insulin therapy, ketoacidosis is rare and these patients rarely, if ever, exhibit circulating glutamic acid decarboxylase (GAD) antibodies.[16] Mitochondrial diabetes can be accompanied by other disorders, such as cardiomyopathy, short stature, and central or peripheral nervous system involvement, including sensorineural deafness, myopathy, encephalopathy, visual failure (retinitis pigmentosa, optic atrophy), with risk of stroke, seizures, and dementia.[14,17]

Proof of the mitochondrial origin of this form of diabetes stems from genetic analysis. The diagnosis is usually made by genetic demonstration of mtDNA mutations in PCR-amplified DNA from peripheral blood leukocytes (PBL) or preferably buccal mucosal cells. The level of heteroplasmy can sometimes be low in these PBL cells and has a tendency to decline with aging, which can make the diagnosis difficult. In a very large fraction of patients with mitochondrial diabetes, there are heteroplasmic mutations that can often be detected in the mtDNA, particularly in genes encoding tRNAs (Figure 8.1). Heteroplasmy can be quite low in patients with mitochondrial diabetes, and there does not appear to be a strong

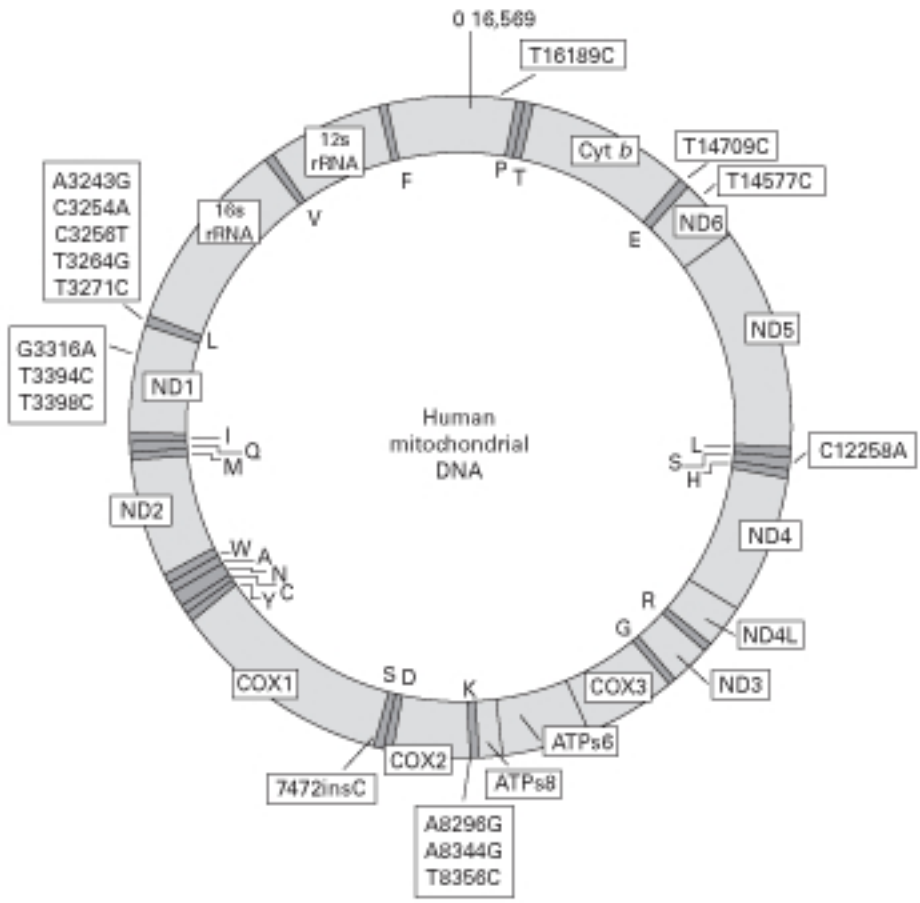

Figure 8.1 The human mitochondrial DNA (mtDNA) and mtDNA mutations associated with diabetes. (Modified from Maechler and Wollheim, *Nature* 2001.[21])

relationship between the level of heteroplasmy in circulating cells and the severity of the disease.[18] However, the age of onset of diabetes may be correlated to the level of heteroplasmy.[18]

The leucyl-tRNA^{UUR} substitution of A for G at nucleotide 3243 (A3243G), traditionally associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), seems to be particularly associated with diabetes, and is the causative lesion in a distinct syndrome known as maternally inherited diabetes and deafness (MIDD).[19] At least seven other mtDNA mutations (Figure 8.1), particularly in tRNA genes of the mitochondrion, can be associated with diabetes, although A3243G-appears to be the most common form.[20,21] mtDNA deletions can also be associated with diabetes.[22]

MIDD is characterized by diabetes as well as by a neurosensory hearing loss (as reflected by a reduced perception of high-tone frequencies > 5 kHz) that typically precedes diabetes. Other co-morbidities have also been reported, including gastrointestinal abnormalities (e.g. dysmotility), cardiomyopathy, renal dysfunction, and macular

pattern dystrophy.[16,23,24] A3243G is generally regarded as a mutation, though in East Asians this variant is actually quite common and is formally a polymorphism.[23] Diabetes or impaired glucose tolerance associated with the A3243G mutation becomes clinically manifest typically in the early 30s. A study of Dutch individuals with the A3243G mutation revealed that by age 70 nearly all individuals have impaired glucose tolerance or diabetes.[18]

Patients with mitochondrial diabetes may or may not initially be insulin-requiring, and can initially be treated with sulfonylurea agents or thiazolidinediones (TZDs). These patients can also be treated with metformin, although this is discouraged given the theoretical risk of lactic acidosis. Insulin treatment is typically required later as the disease progresses.

A major unanswered question is how mutations in mtDNA, particularly in the leucyl-tRNA^{UUR} gene, can give rise to diabetes. Defects in insulin production as well as insulin resistance have been reported in patients with A3243G-associated diabetes.[18] Most studies to date suggest that the defect of the A3243G mutation initially gives rise to islet cell dysfunction and an initial defect in insulin secretion.[20,25,26]

In vitro studies have suggested that a high fraction of heteroplasmy can result in decreased oxidative phosphorylation (OXPHOS) capacity in cybrid cell lines.[27] The A3243G mutation itself is believed to result in dimerization of leucyl-tRNA^{UUR} and decreased aminoacylation.[28] The precise biochemical consequences are not known but in cybrid cells it appears that accumulation of the mutation leads to decreased oxygen consumption and ATP production. In islet cells, this may have the consequence of lowering the ATP/ADP ratio, which could lead to decreased insulin secretion. In addition, alterations in the electron transport chain may give rise to increased reactive oxygen species (ROS), which may lead to increased apoptosis and further decline of islet cell function. This hypothesis is consistent with the age-dependent decline in β-cell function observed with the A3243G-mutation, and is consistent with post-mortem studies which have demonstrated decreased islet cell mass in β-cells as well as in the glucagon producing α-cells in patients with the mutation.[29] The simultaneous loss of glucagons may account for the fact that these patients rarely suffer from diabetic ketoacidosis (DKA).

Mendelian disorders characterized by mitochondrial dysfunction and diabetes

It is worthwhile noting that several Mendelian disorders associated with mitochondrial dysfunction often exhibit diabetes.

Wolfram syndrome (also known as DIDMOAD, or diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) is an autosomal recessive disorder due to mutations in the *WFS1* gene on chromosome 4. The disorder is characterized by type 1 diabetes in association with optic atrophy. WFS1 encodes a transmembrane protein called wolframin, which appears to be localized to the ER and to the mitochondrion, perhaps as a regulator of cellular calcium.[30]

Friedrich's ataxia is an autosomal recessive disorder characterized by a progressive neurodegenerative disease characterized by cerebellar ataxia, dysarthria, nystagmus, and cardiomyopathy. About 20% of patients with this disease also develop insulin resistance sometime during their lifetime. The disease is associated with an expanded trinucleotide repeat in the frataxin gene, whose gene product is a mitochondrial protein directing iron–sulfur-cluster assembly.

Patients with familial amyotrophic lateral sclerosis (ALS) often have mutations in genes encoding ROS scavenging enzymes of the cell. In a number of small studies it has been shown that these patients suffer from impaired glucose tolerance and diabetes mellitus.[31]

Finally, Huntington's disease is another disorder that is characterized by degeneration of the basal ganglia as well as by chorea and dementia. In humans and in mouse models, diabetes mellitus is frequently seen. These patients typically have insulin deficiency, but later, they also have measurable insulin resistance.[32] While the exact function of huntingtin is still not known, recent work has suggested that the mutant protein may interfere with mitochondrial biogenesis via the transcriptional co-activator PGC-1α (D Krainc et al., unpublished results).

Mitochondrial contribution to the common form of diabetes

Based on the above studies it is clear that mutations in mtDNA or in nuclear genes can give rise to syndromes that can be characterized, in part, by diabetes. However, there is mounting evidence that even type 2 diabetes may stem from defects in mitochondrial function. It has long been appreciated that the inheritance of type 2 diabetes shows an excess of maternal transmission[33,34] – this could be due to intrauterine effects, imprinting, or possibly involvement of mtDNA. The two hallmark features of the common form of diabetes, impaired insulin secretion and reduced insulin action (insulin resistance), may have mitochondrial etiologies. Here, we review the evidence suggesting mitochondrial involvement in both of these key processes.

Mitochondria and β-cell function

Blood glucose is carefully regulated by insulin secretion from pancreatic β-cells (Figure 8.2). Glucose equilibrates across the plasma membrane and is phosphorylated by glucokinase to produce glucose-6-phosphate. This step regulates the rate of glycolysis and the production of pyruvate. When blood glucose levels are high, the level of glycolysis in the β-cell is high. Pyruvate then enters the TCA cycle, situated in the mitochondrion, which produces NADH and FADH. These reducing equivalents then drive the electron transport chain, producing ATP. The increased ATP/ADP ratio causes the closure of the plasma membrane K_{ATP} channels, allowing the opening of voltage-sensitive Ca^{2+} channels, similar to those found in other excitable cells. The increase in calcium then causes the release of insulin-containing secretory granules.

Hence, mitochondrial metabolism is able to directly link plasma glucose levels with insulin release, a process known as metabolism–secretion coupling. Several decades ago it was established that mitochondrial dysfunction results in impaired glucose-stimulated insulin secretion. Lowering oxygen levels and poisoning the electron transport chain with inhibitors can block this response. Cells in which mtDNA has been depleted, ρ^0 cells, are viable but exhibit impaired insulin secretion. Interestingly, agents that raise calcium are still able to induce insulin release in such cells, suggesting that the defect is in the mitochondrion. In fact, replenishment of the cells with mtDNA restores glucose-induced insulin release.[35]

Genetic studies in mice, too, have clearly shown that β-cell dysfunction can result from mitochondrial dysfunction. Tfam knockout mice, in which the mitochondrial transcription factor Tfam has been disrupted, exhibit a diabetic phenotype, and the islets exhibit decreased OXPHOS activity and glucose-induced insulin secretion.[36]

Mitochondrial ROS generation and scavenging may serve as a link between organelle dysfunction and β-cell demise. Excess glucose and lipids, which can lead to increased ROS, are known to be toxic to β-cells.[37] It is believed that the β-cell guards against this possibility through uncoupling protein 2 (UCP2), an inner membrane mitochondrial protein that can dissipate the proton motive force and that is activated by superoxide. Genetic and environmental factors may result in increased β-cell ROS generation, which may lead to cellular apoptosis and loss of β-cell mass.

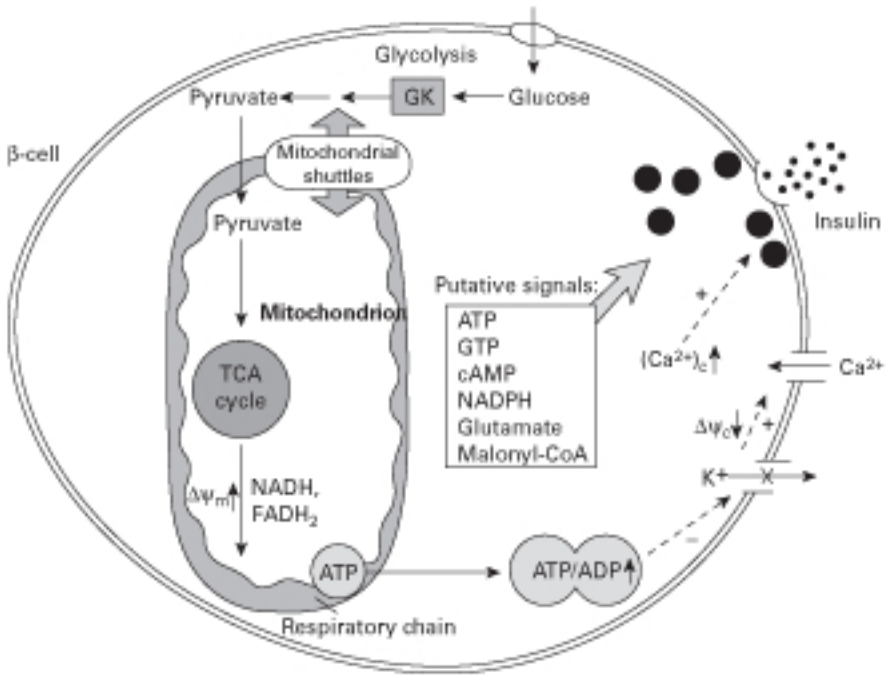

Figure 8.2 Role of mitochondria in β-cell function. The mitochondrion plays a central role in linking metabolism to insulin secretion. Glucose enters the pancreatic β-cell and is metabolized to pyruvate via cytosolic glycolysis. Via the mitochondrial oxidative phoshorylation (OXPHOS) system, pyruvate is oxidized to generate ATP from ADP. This results in a net increase in the cytosolic ATP/ADP ratio, which closes the K_{ATP} channels and depolarizes the β-cell. Depolarization then activates voltage-gated calcium channels which allow calcium influx into the β-cell, leading to the secretion of insulin granules. (Modified from Maechler and Wollheim, *Nature* 2001.[21])

Insulin resistance and mitochondria

For years, it has been appreciated that insulin resistance in skeletal muscle, fat, and liver is one of the hallmark features of diabetes. In high-risk individuals, deposition of fat in muscle and liver precede insulin resistance, as the first detectable feature of the disease.[12,38] While a number of pathways have been implicated in cellular or animal models of insulin resistance, none of these pathways has been shown to be consistently altered in the common form of diabetes.

Several recent genomic approaches have pointed to impaired mitochondrial biogenesis (Figure 8.3) as a common feature underlying insulin resistance.[8,9,39] These studies used DNA microarrays to profile the skeletal muscle of individuals with varying levels of insulin resistance and independently reached the conclusion that in individuals with diabetes, there is a reduced expression of the nuclear genes encoding mitochondrial proteins, in particular, those encoding components of oxidative phosphorylation. Mootha et al. showed that in Northern Europeans the expression of OXPHOS genes is reduced not only in diabetics, but also in individuals with impaired glucose tolerance.[8] Moreover, the expression of OXPHOS genes is highly correlated with VO_2max in all individuals, which has previously been shown to be an extremely strong marker of insulin sensitivity. Patti et al.

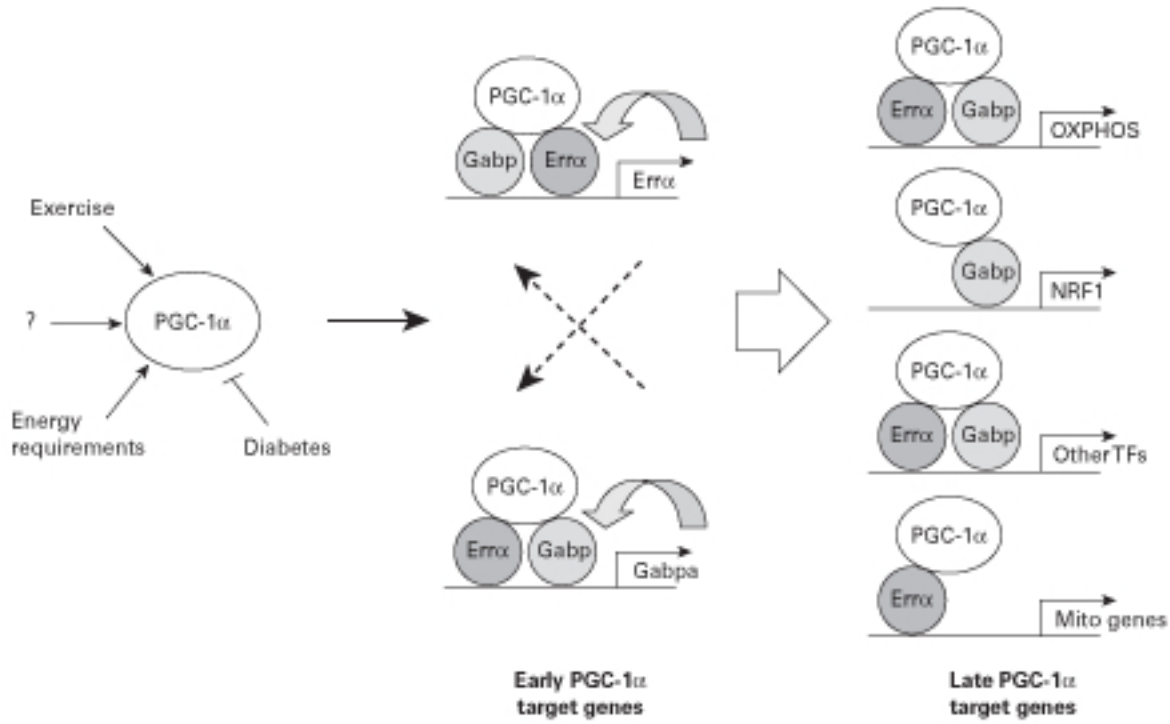

Figure 8.3 Overview of mitochondrial biogenesis. The transcriptional co-activator PGC-1α is a key regulator whose expression levels are controlled by cold, adrenergic inputs, exercise, nutrient status, and diabetes. As PGC-1α levels rise, it partners with the transcription factors ERRα and GABPA/B to co-activate these transcription factors via a double-positive feedback loop. Together, these three factors then partner to stimulate the transcription of a number of genes, including nuclear-encoded members of OXPHOS and other mitochondrial proteins.[40] In addition, this circuit leads to the stable rise of nuclear NRF-1, a transcription factor which is involved in the regulation of the mitochondrial transcription factor TFAM, which is imported into the mitochondrion to promote mtDNA replication.[76] PGC-1 = peroxisome proliferator-activated receptor-γ co-activator 1, ERRα = estrogen related receptor alpha; GABP = GA binding protein A; NRF1 = nuclear respiratory factor-1

examined Mexican Americans and showed that the expression of OXPHOS genes is also reduced in the muscle of healthy, first-degree relatives of individuals with type 2 diabetes.[9] Both of these studies hence suggest that reduced OXPHOS expression is a phenotype appearing relatively early in the development of type 2 diabetes.

Mootha et al. further showed that observed changes appear to lie downstream of the transcriptional co-activator PGC-1α, which has been shown to be a master regulator of mitochondrial biogenesis. Because the expression of PGC-1α is also reduced in these patients, it appears that in the common form of diabetes, in Northern Europeans as well as in Mexican Americans, there is a PGC-1α-dependent decrease in mitochondrial biogenesis. A recent study has further dissected the pathway of mitochondrial biogenesis and suggests that the orphan nuclear receptor ERRα, the ETS transcription factor GABPA/B, and PGC-1α may form a regulatory switch that lies upstream of NRF1 and other transcription

Box 8.1 Microarray technology

The availability of the complete sequence of the human genome means that, in principle, we know the entire set of encoded human mRNAs. We should therefore be able to ask if the expression of these mRNAs is altered under various conditions (e.g. glucose-rich vs glucose-free medium, or in normal vs diseased tissue). From a practical standpoint, Northern blot analysis can examine only a handful of mRNAs at a time, and the various subtractive hybridization methods in use are time consuming.

The development of DNA microarrays, also known as gene chips, has solved this problem. Short oligonucleotides (20–50 nt in length), each with a defined and unique sequence corresponding to each mRNA, is arrayed on a grid (Figure B.1).[1] The grid is about the size of a postage stamp, but can contain thousands of 'cells,' each with an oligonucleotide representing one specific gene or mRNA. In a modification of this method, hundreds of full-length cDNAs representing individual mRNAs are 'spotted' on a glass slide and are analyzed in a similar manner.[2]

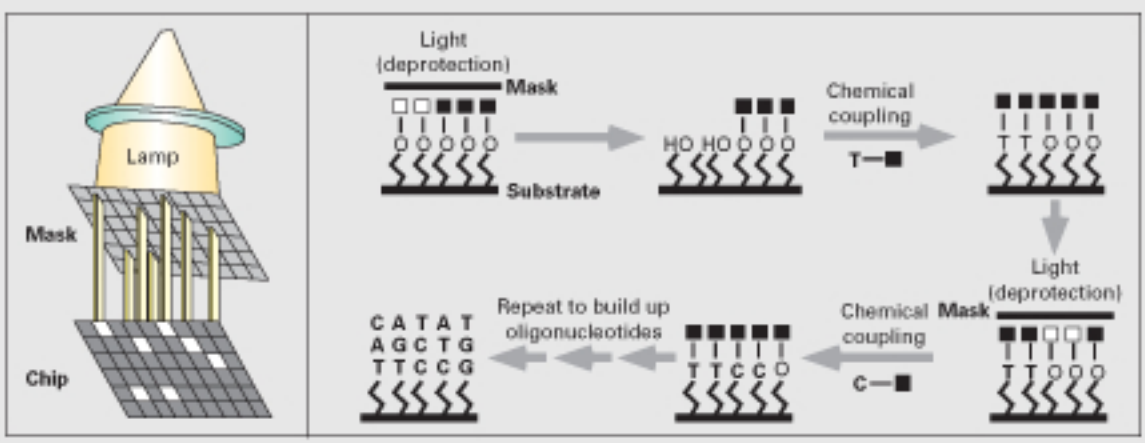

Figure B.1 Microarray technology. **(A)** Using microlithography to make a chip. **(B)** The nucleotide sequence on the array is 'built up' in a series of light-catalyzed steps that successively protect and deprotect the nucleotides in a controlled manner. Adapted from reference 1 with permission

The chip is 'queried' by hybridizing fluorescently labeled cDNAs derived from mRNAs from the two sources under comparison (e.g. red cDNA probes from mRNA derived from normal tissue and green probes from diseased tissue mRNA), and comparing the colored signals derived from each source (Figure B.2). Thus, one can determine in a single experiment which genes are up-regulated (i.e. red signal predominates), which are down-regulated (i.e. green signal prominates), and which are unchanged (both red and green signals are equal in intensity, giving a yellow signal).

Microarray analysis can aso be used to develop 'functional maps,' in which each mRNA is a node in a network, with mRNAs 'pointing' towards or away from other mRNAs, depending on whether they are upstream or downstream of each other in a pathway.[3]

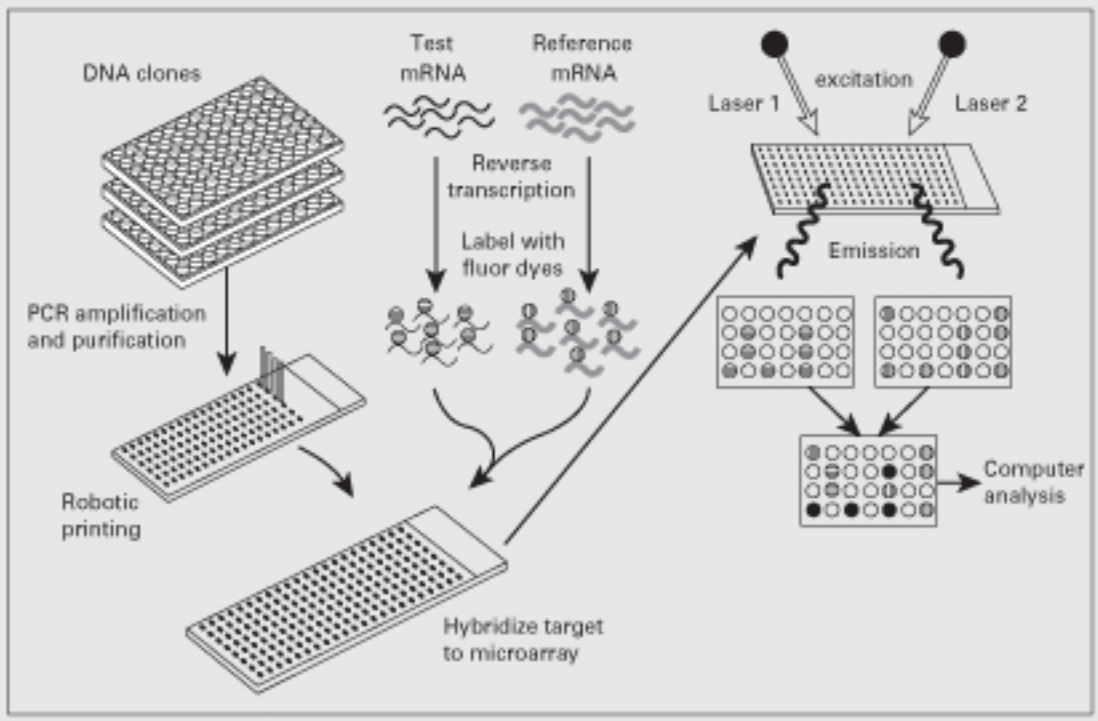

Figure B.1 Microarray analysis. Adapted from reference 2, with permission

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factors in mediating mitochondrial biogenesis[40] – this regulatory circuit may represent a novel target for antidiabetic medications.

Functional studies using very different approaches have also suggested that reduced OXPHOS activity in muscle may represent a signature feature of diabetes. Kelley and colleagues have examined skeletal muscle from individuals with impaired glucose tolerance as well as diabetes and found altered mitochondrial morphology as well as OXPHOS activity.[12] Petersen and Shulman have used[31] P-NMR-based measurements of OXPHOS activity in vivo to demonstrate that diabetics and IGT individuals have reduced OXPHOS capacity. Moreover, they showed that the healthy first-degree relatives of individuals with DM2 also have reduced OXPHOS activity.[11] These functional studies complement the genomic studies and further establish reduced mitochondrial biogenesis as a feature of diabetes.

Box 8.2 Cybrid technology

There is no known way to transfect human mitochondria with exogenous DNA in a stable and heritable manner. However, one can transfer patient mitochondria containing mutated mtDNAs from one cell to another, and then study these mutated mtDNAs in a neutral nuclear background. This is accomplished by making cytoplasmic hybrids, or 'cybrids' in which patient cells devoid of their nuclei (cytoplasts) but still containing mitochondria (cytoplasts) are fused with cells containing mitochondria that are devoid of their endogenous mtDNA (called ρ^0 cells, based on the yeast nomenclature.[1]

How are ρ^0 cells made? Typically, a transformed cell line – most commonly a human osteosarcoma line called 143B – that is deficient in thymidine kinase activity (TK^-) becomes ρ^0 following long-term exposure to ethidium bromide, an inhibitor of mtDNA replication that is preferentially taken up by mitochondria, owing to its high membrane potential (EtBr is a charged molecule). The ρ^0 line is auxotrophic for pyrimidines (such as uridine), due to the loss of a functional respiratory chain (because without a respiratory chain, the mitochondrial protein dihydroorotate dehydrogenase, a key enzyme in uridine biosynthesis, cannot function). For reasons that are less well understood, the cells are also auxotrophic for pyruvate.

This auxotrophy provides two selection schemes for the repopulation of these cells by exogenous mtDNA, based on complementation of the metabolic defects with exogenous mitochondria (and mtDNAs). The ρ^0 cells are repopulated by forming 'cytoplasmic hybrids' (cybrids) between the ρ^0 cells and cytoplasts (enucleated cells) from an mtDNA donor cell line. After cell fusion, cells are plated in medium containing bromodeoxyuridine (BrdU) and lacking either pyruvate or uridine. These selective media permit only the growth of ρ^0 cells which had fused with cytoplasts containing functional mitochondria, because the ρ^0 cells (which have defective thymidine kinase (TK) activity, another enzyme required for pyrimidine biosynthesis) are not able to grow in the absence of uridine or pyruvate, and TK^+ donor cells are not able to grow in the presence of BrdU.

Once the cybrids are made, they can be grown as cellular clones. Because the pool of cybrid cells reflects the (Gaussian) distribution of heteroplasmy represented in the original population of patient cells, one can isolate cybrid clones harboring varying proportions of mutated mtDNAs, ranging from 0% mutant (100% wild-type (homoplasmic)) to 100% mutant (i.e. 0% wild-type (also homoplasmic)), plus anything in between (i.e. heteroplasmic). If one cannot find homoplasmic mutant clones, a clone with a high % heteroplasmy can be subjected to a second round of EtBr treatment, in which the mtDNA copy number is reduced from about 10 000 copies/cell to less than 50 copies/cell.[2] At that point, the EtBr is removed and the cells are allowed to repopulate their mtDNAs, resulting in a new population of cells with a skewed proportion of mtDNAs, including cells that are both homoplasmic wild-type and homoplasmic mutant.

Cybrid technology has been used successfully to study many pathogenic mtDNA mutations, including those causing MELAS, MERRF, NARP/MILS, and KSS.

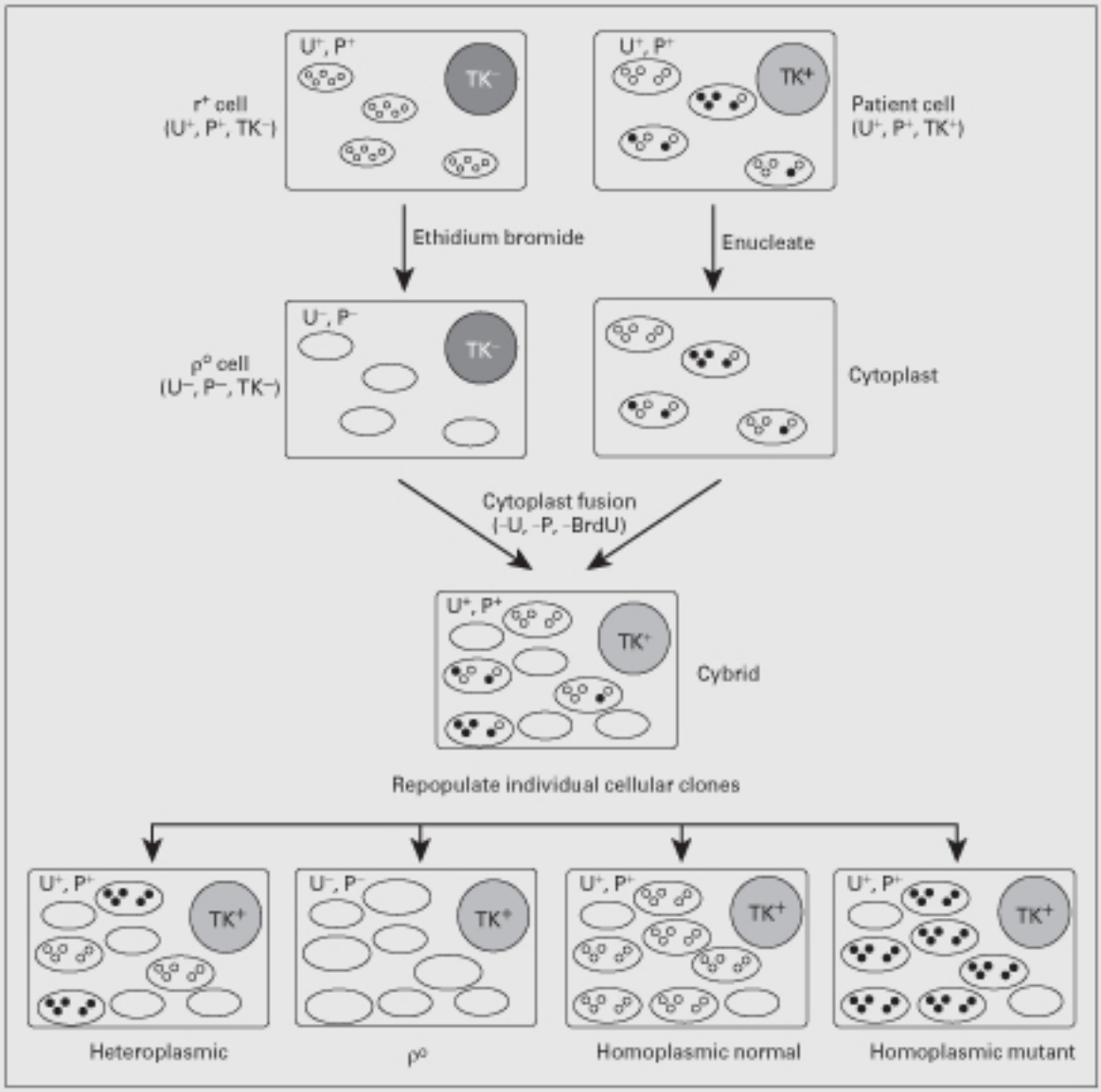

Figure B.1 Making ρ° cells and cybrids.

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Exactly how reduced OXPHOS capacity is related to insulin resistance is currently not known. Specifically, we currently do not know whether a common signal leads simultaneously to reduced OXPHOS expression and insulin resistance, or whether there is a causal relationship between OXPHOS capacity and insulin resistance. Insulin resistance is characterized by impaired insulin-stimulated glucose transport, phosphorylation, and glycogen synthesis. Enhanced accumulation of triglycerides in muscle correlates strongly with impaired insulin-stimulated glucose uptake. A prerequisite for enhanced accumulation of intramyocellular lipids is that their oxidation is impaired, and in fact, impaired lipid oxidation has been demonstrated both in the fasting state as well as during insulin stimulation in patients with type 2 diabetes. Reduced mitochondrial activity could theoretically lead to impaired fat oxidation, leading to accumulation of long-chain fatty acids and increased formation of diacylglycerol (DAG). Through involvement of PKC or the IKkβ/NFkβ complex and serine phosphorylation of key targets in the insulin signaling pathway, such fatty acid accumulation could lead to skeletal muscle insulin resistance.[41] George Thomas' group has recently shown that the signaling molecule S6 kinase (S6K), an integrator of nutrient sensing, signaling, and protein synthesis, may play a key role in integrating nutrient levels with mitochondrial biogenesis and insulin receptor activity.[42]

Mitochondrial dysfunction in diabetes: cause or consequence?

The question still unanswered is whether inherited or acquired mitochondrial dysfunction represents the cause or the consequence of type 2 diabetes. Functional, epidemiological, and clinical studies favor the causal hypothesis.

First, the decreased expression of PGC-1α and OXPHOS genes is seen already in individuals with impaired glucose tolerance or in healthy first-degree relatives of patients with type 2 diabetes.[8,9] Also, first-degree relatives of patients with type 2 diabetes show impaired ATP synthesis in skeletal muscle as measured by NMR spectroscopy.[11]

Second, from an epidemiological standpoint, it is worthwhile recalling that even the common form of diabetes tends to exhibit excess maternal transmission.[34] In fact, a number of small studies have suggested that mtDNA haplotypes can contribute to the risk of diabetes.[43]

Third, a number of genetic studies have shown that polymorphisms in several genes are associated with the common form of diabetes. Perhaps the strongest result is from PPAR-γ, a nuclear receptor that is the target of the thiazolidinediones (TZDs). The Pro12Ala variant, which is found in 10–15% of the Caucasian population, is associated with a 15% decreased risk of diabetes.[44] Variation in the HNF4α promoter is also associated with the common form of diabetes.[45,46] These nuclear receptors are all transcriptional partners of PGC-1α, a master regulator of mitochondrial biogenesis.[47] In fact, variation in PGC-1α has also been associated with diabetes, though these have not been broadly replicated.[48]

Finally, clinical and pharmacologic studies strongly support the notion that mitochondrial biogenesis can contribute to diabetes. It has long been appreciated that the best non-pharmacologic intervention for improving insulin resistance is exercise. The recent Diabetes Prevention Program Trial in patients with type 2 diabetes confirmed that exercise was the most effective way to prevent diabetes in those with impaired glucose tolerance.[49] Exercise is known to increase mitochondrial biogenesis in skeletal muscle and concomitantly improve total body VO_2max, which is itself a marker for insulin resistance. The TZDs represent a category of drugs that improve insulin resistance. Recent studies have shown that they serve as a synthetic agonist for the nuclear hormone receptor PPARγ, and one of the effects is to increase mitochondrial biogenesis in fat

and possibly in muscle.[50] Recent studies have also shown that high intake of caffeine can reduce one's lifetime risk of developing diabetes,[51] and caffeine has been shown in vitro to increase mitochondrial biogenesis.[52] In addition, the lipodystrophy seen in patients with human immunodeficiency virus infection, appears to be due to the mitochondrial toxicity of highly active antiretroviral therapy.[53]

Taken together there is biochemical, genetic, and clinical support for a key role for mitochondrial biogenesis in diabetes. Additional studies are needed to determine if and how mitochondrial diabetes resulting from mtDNA mutations may be related to the expression phenotype seen in the common form of the disease.

Other endocrine disorders in mitochondrial disease

Here, some of the other endocrine manifestations in patients with mtDNA disorders are reviewed.

Short stature

Short stature is defined as a standing height more than two standard deviations below the mean for age and gender. Short stature may result from endocrine disease, systemic illness (e.g. chronic renal failure, malnutrition), or primary skeletal disorders (e.g. achondroplasia, SHOX haploinsufficiency). The endocrine causes of short stature include impaired growth hormone (GH) secretion or action, hypothyroidism, and glucocorticoid excess. Short stature is frequently seen in patients with mtDNA defects, with an estimated 35% of all such patients so affected.[4,54] It can be seen in patients with MELAS, myoclonus epilepsy and ragged-red fibers (MERRF), progressive external ophthalmoplegia (PEO), and Kearns–Sayre syndrome (KSS). Of note, short stature can also be seen in patients with mitochondrial diseases due to mutations in nuclear genes, as in Barth syndrome.[55]

The etiology of the short stature in mitochondrial disorders is varied. Matsuzaki et al. reported two girls with MELAS in whom they were able to establish a diagnosis of growth hormone-releasing hormone deficiency.[56] In another study, patients with the A3243G mutation underwent growth hormone provocation studies, which revealed deficient pituitary growth hormone secretion.[57] Quade and colleagues carefully evaluated 21 patients with PEO and found six of them had short stature, possibly secondary to growth hormone deficiency.[58] Last, more than one disorder can account for short stature in patients with mitochondrial disease as was demonstrated by a girl with MELAS with both growth hormone deficiency and central hypothyroidism.[59] Short stature is a common symptom of mitochondrial disease, whether a mitochondrial or nuclear defect, and can be due to more than one etiology, the most common of which appears to be GH deficiency. Investigations into the clinical effectiveness of GH supplementation are limited. Although there is no established therapy for short stature in patients with mitochondrial disease, there are reports suggesting that in patients with documented GH deficiency, replacement therapy can have a benefit.[56,60]

Because of the prevalence of this disorder and the potential therapy available, critical evaluation of possible endocrine causes and therapies is needed.

Gonadal dysfunction

Patients with gonadal disorders can present with a variety of signs and symptoms. Males may present with cryptorchidism, lack of or delay in puberty, low libido, and erectile dysfunction. In females, the manifestations include delayed puberty, late menarche, primary or secondary amenorrhea, or oligomenorrhea. The etiology may be as a result of hypothalamic, pituitary, or gonadal (ovaries and testes) disease. Gonadal dysfunction appears to frequently accompany mtDNA-based disorders

and has been described in patients with PEO, MELAS, and MERRF.[58,61]

In a study of 21 patients with PEO, Quade et al. found that 38% exhibited symptoms, physical exam signs, or laboratory evidence of a reproductive disorder.[58] Chen et al. reviewed six patients with MELAS and MERRF, and five had evidence of gonadal dysfunction either by history or physical examination.[61] Symptoms included delayed puberty, primary amenorrhea, or secondary amenorrhea in women and delayed puberty and erectile dysfunction in men. Gonadotropin levels and luteinizing hormone-releasing hormone stimulation testing suggest hypothalamic or pituitary dysfunction. In fact, an autopsy report of a patient with MELAS due to a mitochondrial tRNA Leu(UUR) mutation revealed that the hypophysis had the highest level of heteroplasmy.[62]

In addition to the studies described above, there are a few case reports describing testosterone insufficiency in patients with mtDNA mutations.[63]

Because of the small number of studies to date, it is difficult to determine the actual prevalence of reproductive dysfunction in patients with mitochondrial disease as well as the site of the defect (hypothalamic, pituitary, or gonadal). No studies have systematically examined the safety and efficacy of testosterone or estrogen replacement therapy in patients with mitochondrial disease. It is interesting to postulate whether or not gonadal disorders have mitochondrial pathophysiologic correlates in the same manner as diabetes.

Other endocrine manifestations

A variety of other endocrine disorders have been described in case reports or small case series of mtDNA diseases. These endocrine disorders include hypoparathyroidism, thyroid disease (hypothyroidism or hyperthyroidism), and adrenal disease (adrenal insufficiency or hyperaldosteronism).

Hypoparathyroidism is characterized by deficient parathyroid hormone production. Symptoms can range from subclinical disease to parasthesias to seizures, depending on the degree of resulting hypocalcemia. Hypoparathyroidism has been described in patients with mitochondrial disorders, especially those with KSS and PEO.[64,65]

Hypothyroid symptoms include cold intolerance, fatigue, weight gain, coarse hair, and constipation. Hyperthyroid symptoms include heat intolerance, palpitations, weight loss, and diarrhea. Symptoms can be extremely mild to very severe depending on the degree of dysfunction. Thyroid dysfunction, both hypothyroidism and hyperthyroidism, has been reported in patients with mitochondrial disease[66,67] and was found in up to 17% of patients with respiratory chain dysfunction in one study.[4] It does not appear to be a feature of mtDNA disorders characterized by deletions.[54,58,68]

Symptoms of adrenal insufficiency include nausea, fatigue, weight loss, and orthostasis. Adrenal insufficiency has been described in patients with mitochondrial disease.[69–72] Hyperaldosteronism, which can present as hypertension, has also been described in patients with mitochondrial disease.[54,73] As with many of the other endocrinopathies, the actual prevalence of adrenal disease in these patients is not known.

Clinical evalution and treatment considerations

At present, we advocate that all clinicians caring for patients with mitochondrial disease be aware of the reported endocrinopathies that may appear in such patients. All patients should be assessed for possible diabetes, short stature, gonadal dysfunction, thyroid dysfunction, hypoparathyroidism, and adrenal insufficiency (Table 8.1).

An initial history and physical exam ought to explore these possible features. An initial history must include assessment for polydipsia, polyuria, and weight loss as initial signs of diabetes mellitus. Questions should be asked regarding regularity of menses in women and erectile function and libido

Table 8.1 Endocrine manifestations in mitochondrial disorders

Endocrinopathy	Signs and symptoms	Screening tests
Diabetes	Polyuria, polydipsia, polyphagia, weight loss	Fasting blood sugar
Short stature	Short stature for gender and age	Plot height on growth chart: if 2 SD below mean: IGF-1, IGF-BP3, bone age, GH provocative testing
Hypogonadism	Poor development of secondary sexual characteristics, infertility, delayed puberty, amenorrhea	In men: morning total testosterone, LH, FSH In women: estradiol, LH, FSH
Hypoparathyroidism	Tetany, parasthesias, seizures, cramps	Calcium, albumin, phosphorus, parathyroid hormone (PTH)
Hypothyroidism	Weight gain, fatigue, cold intolerance, constipation, dry skin, hair loss, menstrual irregularities	Thyroid-stimulating hormone (TSH), free thyroxin (free T4)
Adrenal insufficiency	Orthostasis, fatigue, hyponatremia, hyperkalemia	Morning cortisol: if < 18 μg/dL, then cosyntropin stimulation test

SD = standard deviation; IGF-1 = insulin-like growth factor-1; IGF-BP3 = insulin-like growth factor binding protein 3; GH = growth hormone; LH = leutenizing hormone; FSH = follicle-stimulating hormone.

in men. Symptoms of thyroid disease such as heat or cold intolerance, weight loss or gain, change in skin or hair texture, constipation and diarrhea should be elicited. A diagnosis of hypoparathyroidism should be considered when patients describe muscle cramping or paraesthesias.

Physical examination ought to include a careful height measurement, thyroid examination, assessment of secondary sexual characteristics, and assessment of Chvostsek's and Trousseau's signs.

An initial laboratory evaluation ought to include a fasting glucose level. In addition, calcium, albumin, phosphate, parathyroid hormone (PTH), thyroid-stimulating hormone (TSH) and free thyroxine (free T4) levels should be ordered to assess for hypoparathyroidism and thyroid disease. If gonadal dysfunction is suggested by the history or physical exam, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) should be ordered in addition to an estradiol level in women and an early morning total testosterone level in men. If orthostasis, nausea, or unexplained weight loss are present, then the patients should be assessed for adrenal insufficiency with a morning cortisol or cosyntropin stimulation test. If any of the components of the history, physical, or screening endocrine labs are suggestive of diabetes, short stature, gonadal dysfunction, thyroid disease, hypoparathyroidism or adrenal insufficiency, we advocate early referral to an endocrinologist for careful diagnostic studies followed by treatment initiation (Table 8.1).

Treatment

For patients with mtDNA-based diabetes, it is likely that the mainstay of therapy will involve sulfonylureas, diet, and insulin therapy. We have found that these patients can also benefit from insulin sensitizers, in particular TZDs. Future

studies may determine whether specific forms of disease may benefit from specific therapies. Because metformin has been associated with lactic acidosis in isolated cases, we currently prefer avoidance of this drug, especially in patients with MELAS. While carnitine, antioxidants and CoQ10 are often prescribed to patients with mtDNA disorders, there are no studies to date that provide convincing evidence that they improve diabetes.

With regard to the other endocrinopathies, hormone replacement of the deficient hormone(s) may be needed. Female and male patients diagnosed with reproductive disorders may require estrogen and testosterone replacement, respectively. Patients with hypothyroidism will need to be treated with thyroid hormone replacement, and patients with hypoparathyroidism may need calcium and vitamin D replacement.

Summary

It is clear that a variety of endocrinopathies accompany all varieties of human mitochondrial diseases, those due to mtDNA mutations as well as those due to nuclear defects. Tremendous research has focused on the role of the mitochondria in various forms of diabetes. We anticipate that in the coming years, specific mechanistic links between this organelle and insulin deficiency and insulin resistance will be established.

In patients with mitochondrial disease, however, we are lacking careful and systematic studies of endocrine function. It is important that we first determine the frequency of endocrinopathies in patients with various mitochondrial diseases and how these endocrinopathies manifest in patients with mitochondrial diseases. In addition, studies are needed to determine the etiology of these endocrinopathies and may require careful frequent hormone blood sampling and/or stimulation testing. Last, the efficacy of current endocrine standard treatments must be assessed in these patients.

The potential psychological and physical benefits of hormonal replacement may prove to greatly improve mitochondrial patients' quality of life. Treating short stature and hypogonadism will help with social interactions. Thyroid hormone replacement can influence mitochondrial biogenesis and can directly impact mitochondrial energetics. Normalization of calcium may help with neurologic symptoms, including seizure control, and treatment of adrenal insufficiency may help with fatigue and orthostasis seen in patients. Because the endocrine system is so intimately linked to cellular energetics, careful evaluation and treatment of endocrine dysfunction in patients with mitochondrial disease is extremely important and should be pursued.

Acknowledgments

We thank David Holtzmann, Paul Boepple, Katherine Sims, and Corrine Welt for useful discussions and careful review of this chapter.

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