Insulin Resistance Associated With Maternally Inherited Diabetes and Deafness

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Maternally inherited diabetes and deafness (MIDD) is a form of diabetes associated with mutation of mitochondrial DNA (mtDNA) that occurs in 1% to 2% of individuals with diabetes. Understanding the clinical course and abnormalities in insulin secretion and action in affected individuals should allow better understanding of how this genetic defect alters glucose metabolism. We report the clinical course of three individuals with mtDNA mutations and deafness. Subjects no. 1 and 2 had diabetes not yet requiring insulin therapy, and subject no. 3, the son of subject no. 2, had normal glucose tolerance. Defective oxidative phosphorylation (OXPHOS) based on OXPHOS enzymology of skeletal muscle biopsy of subjects no. 1 and 2 showed activity of less than 5% of the tolerance level in complex III for subject no. 1 and in complexes I, I + III, and IV for subject no. 2. Assessing insulin secretion using insulin response to intravenous glucose and insulin sensitivity based on minimal model analysis of an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT), first-phase insulin secretion was abnormal in subjects no. 1 and 2 and normal in subject no. 3 (AUC, 57, 93, and 1,235 pmol/mL, respectively). In contrast, all three subjects had low insulin sensitivity indices (0.04, 0.14, and 0.27 × 10^{-4} × min/pmol/L, respectively). Subject no. 2, who underwent three FSIGT studies over a 16-month interval, showed transient improvement in insulin release in response to modification of diet and exercise (first-phase insulin AUC, 57 pmol/min *v* 287 pmol/min 10 months later; fasting insulin, 97 pmol/L *v* 237 pmol/L 10 months later), but by 16 months, first-phase insulin release and fasting insulin had decreased (AUC, 64 and 136 pmol/L, respectively) despite higher fasting glucose. We conclude that in our subjects with MIDD, insulin resistance is present and appears to precede defects in insulin release.

HUMAN MITOCHONDRIAL DNA (mtDNA) is a double-stranded, 16,569–base pair circular molecule that encodes for ribosomal and transfer RNAs of mitochondrial protein synthesis and for 13 of approximately 100 polypeptides required for oxidative phosphorylation (OXPHOS). The inheritance pattern is exclusively maternal in humans. Pathologic syndromes linked to mtDNA mutations have been shown to result in abnormalities of OXPHOS function and have been characterized by degenerative abnormalities of the central nervous system, cranial nerves, and muscle.[1] OXPHOS results from the interaction of five protein-lipid complexes for which the polypeptide subunits are encoded by both the nuclear DNA and the mtDNA. Metabolism of fats, proteins, and carbohydrates results in the transfer of electrons to complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), or coenzyme Q10 of OXPHOS. The energy released by the sequential transfer of these electrons to complex III (ubiquinol-ferrocytochrome c oxidoreductase) and complex IV (ferrocytochrome c-oxygen oxidoreductase or cytochrome c oxidase) is used to pump protons into the space between the inner and outer mitochondrial membranes. The energy stored in this proton gradient is then available to complex V (adenosine triphosphate [ATP] synthase) for the synthesis of ATP from adenosine diphosphate (ADP). This process uses approximately 90% of the oxygen delivered to cells and generates over 90% of the ATP requirements for most cells.

Mitochondrial ATP is delivered to the cytoplasm by the inner-membrane protein adenine nucleotide translocase in exchange for cytoplasmic ADP.[1] This microcompartmentation of ATP production within mitochondria allows cells to carefully control ATP availability through a variety of complex mechanisms that include ATP delivery to specific ATP-dependent enzymes.[2] Individuals who inherit a pathogenic mitochondrial mutation can have mild clinical manifestations, absence of significant muscle pathology, and mild, highly variable OXPHOS defects, whereas severely affected individuals generally have more extensive organ involvement, more severe OXPHOS defects, and ragged-red fiber myopathies.[1]

Maternally inherited diabetes and deafness (MIDD) is a syndrome associated with two classes of mutations in mtDNA: complex mtDNA rearrangements and mtDNA point mutations. A family with a complex mtDNA rearrangement consisting of three distinct mtDNA species (a normal 16.5-kb mtDNA, a 6.1-kb deletion mtDNA, and a 22.6-kb duplication mtDNA) has been identified.[3,4] Although this mtDNA rearrangement is rare, an A-to-G point mutation in the $tRNA^{Leucine(UUR)}$ gene at position 3243 of the mtDNA (MTTL1*MELAS3243G) has been reported to occur in approximately 1% of diabetic adults screened for this defect.[5] In addition to diabetes mellitus, this mutation can produce a broad array of clinical manifestations, including mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS).[1] Individuals with MIDD exhibit characteristics of both non–insulin-dependent (NIDDM) and insulin-dependent (IDDM) diabetes mellitus, but the pathophysiologic defects contributing to abnormal glucose are incompletely understood.[6,7]

We previously reported two subjects with MIDD and clinical features typical of IDDM.[3] These individuals were markedly insulinopenic, had onset of diabetes during the

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teenage years, were lean, had a past history of ketoacidosis, and showed minimal C-peptide response to stimulation. However, in both subjects, diabetes was present for over 20 years.

To gain insight into the natural history and pathophysiology resulting in diabetes mellitus associated with mtDNA mutations, affected individuals should be observed during early stages of glucose intolerance. In this study, we describe three well-characterized subjects who harbor pathogenic mtDNA mutations but have recent onset of diabetes (subjects no. 1 and 2) or normal glucose tolerance (subject no. 3), and apply an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) with minimal model analysis to assess insulin secretion and sensitivity.

SUBJECTS AND METHODS

Three subjects with OXPHOS diseases, from two families, were studied. Informed consent was obtained from all patients, and the protocol was approved by the Human Investigations Committee of Emory University.

OXPHOS Investigations

Skeletal muscle biopsies were performed on patients no. 1 and 2. Histology, electron microscopy, and OXPHOS biochemistry were performed as previously described.[8-10] Abnormal OXPHOS specific activity was defined as a value less than the 5% tolerance level of OXPHOS values measured in muscle biopsies obtained from 30 normal subjects who had no prior history of neuromuscular disease. The age range of the normal subjects was 18 to 49 years.

Human Gene Map designations are used to refer to mutations.[11] Detection of the MTTL1*MELAS3243G mutation was performed in patients no. 1 and 2 as previously described.[12,13] Patient no. 1 and her family harbored a previously described complex mtDNA rearrangement.[3,4]

Insulin-Modified FSIGT

Subjects were studied supine after an overnight fast using a FSIGT modified with an insulin bolus as previously described.[14] The mean of blood samples for insulin and glucose obtained at −15, −5, −1, and 0 minutes was measured for baseline values, and then a bolus of glucose as dextrose 50% (300 mg/kg) was given intravenously with blood sampling at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 160, and 180 minutes. Following the 20-minute blood sampling, an intravenous injection of regular human insulin (0.03 U/kg) was administered. Insulin and glucose values were analyzed using the MINMOD computer program (© R.N. Bergman).

Using the first 20 minutes before injection of insulin, study subjects were analyzed for endogenous insulin response. The insulin response from 1 to 10 minutes was used as a measure of first-phase response.

Measurements

Glucose level was measured with the 550 Express Clinical Chemistry Analyzer (Ciba Corning Diagnostics, Medfield, MA). Assay sensitivity is 1.1 mg/dL, intraassay coefficient of variation 2.6% to 1.4%, and interassay coefficient of variation 2.1% to 1.6%.

Serum insulin level was measured by a direct radioimmunoassay using commercially available reagents (Diagnostic Products, Los Angeles, CA). The assay has a sensitivity of 2.5 μIU/mL. The interassay coefficient of variation, calculated at the midpoint of the standard curve, was 12.1% (n = 18 assays), and the intrassay coefficient of variation was less than 5.0%.

Blood and urine organic and amino acid analyses were performed using a Beckman 6300 amino acid analyzer (Beckman Instruments, Palo Alto, CA), a Hewlett Packard (Palo Alto, CA) 5890 gas chromatograph, and a Hewlett Packard 5971 mass spectrometer, respectively.[15,16] All blood was obtained as a morning fasting sample. A total of 36 controls aged 19 to 70 years were used to determined the 5% to 95% tolerance level[17] for blood and urine organic and amino acids.

Hemoglobin A_{1c} was calculated from glycated hemoglobin measurements obtained by the Emory Pathology Reference Laboratory using the Abbott IMx glycated hemoglobin assay (Abbott Laboratories, Abbott Park, IL). This assay has a mean of 5.0% for 147 normals with a range of 4.4% to 6.4%. The midcurve intraassay coefficient of variation was 4.1% and interassay coefficient of variation 4.8%.

Anti–islet cell antibody levels were kindly measured by the Immuno-endocrinology laboratory at the Joslin Clinic (Boston, MA).

Calculations

The area under the curve (AUC) was calculated (subtracting baseline) using the trapezoidal rule.

RESULTS

Patient Description

Subject no. 1. Subject no. 1 was a 27-year-old female member of a previously reported family (patient no. III-1) with MIDD caused by a complex mtDNA rearrangement.[3,4] She was in good health until approximately 20 years of age, when she noted a mild hearing loss that has been slowly progressive over the last several years. An elevated fasting serum glucose was noted at 25 years of age. After participation in this study, she began insulin therapy. The only other disease manifestation noted was mild to moderate fatigability with activity. Except for the hearing loss, her physical examination was normal.

At 26 and 27 years of age, blood and urine organic acids, amino acids, and L-carnitine levels (Emory Genetics Laboratory, Emory University) were normal. Both assessments were performed before beginning insulin therapy. At 26 years of age, blood electrolytes and chemistries were normal, except for a fasting serum glucose of 133 mg/mL and a mildly decreased serum CO_2 of 23 mEq/L (normal, 25 to 33). Anti–islet cell antibodies were negative.

Subject no. 2. Subject no. 2 was the 38-year-old mother of a child with MELAS (subject no. 3). Her disease manifestation was hearing loss that began at approximately 25 years of age. The hearing loss progressed slowly, and at about 34 years of age, she required bilateral hearing aids. At 36 years of age, she was hospitalized for a hysterectomy and was noted to have an elevated blood glucose. Diabetes mellitus was diagnosed, and she was started on glyburide. She remained on glyburide for 2 years and was started on insulin at 38 years of age, a few months after the last FSIGT study. Ophthalmologic examination, Goldman visual fields, and an electroretinogram were normal, with no evidence of pigmentary retinal degeneration and no evidence of dia-

betic retinopathy. The physical examination was normal except for the presence of bilateral hearing loss.

Routine hematology profile, thyroxine and thyrotropin levels, electrocardiogram, echocardiogram, electroencephalogram, and pattern-reversal visual evoked response were normal. No antibodies were detected to islet cells, mitochondria, or cardiolipin. Blood electrolytes and chemistries were normal, except for a fasting serum glucose of 191 mg/mL.

At 36 years of age, fasting blood and 24-hour urine collected for quantitative organic acid and amino acid analyses were normal. At 38 years of age, the alanine level had increased and was 648 μmol/L in blood (95% tolerance level, 635) and 722 nmol/mg creatinine in urine (95% tolerance level, 530). Blood and urine organic acid analysis was normal. No abnormalities in blood or urine levels of acetoacetate or β-hydroxybutyrate were detected by these analyses. L-Carnitine quantitation (Emory Genetics Laboratory, Emory University) and acyl-carnitine quantitation by fast atom bombardment (Pediatric Genetics Laboratory, Duke University) were normal in blood and urine. Analysis of this subject's mtDNA revealed that she harbored the MTTL1*MELAS3243G mutation.

Subject no. 3. Subject no. 3 was a 17-year-old male with MELAS. The patient was hypotonic at birth and had delayed cognitive and motor development. He sat at 8 months, crawled at 10 months, and walked at 16.5 months of age. At 10 years of age, he began experiencing his first cerebrovascular symptoms. His first episode was characterized by a severe headache, obtundation, and a left hemiparesis. These manifestations resolved. His physicians believed he experienced an unwitnessed seizure and treated him with valproic acid. At 11 years of age, he experienced a generalized seizure with subsequent right hemiparesis that resolved over 3 days. He was treated with carbamazepine and had no further cerebrovascular symptoms. At 13 years of age, he experienced a generalized seizure, and 2 months later he had a right focal seizure with subsequent generalization that was accompanied by a persistent visual-field defect. Head magnetic resonance imaging revealed a left occipital infarct. Cerebral angiogram was normal. His examination showed a normal height, microcephaly, hearing loss, a mild pigmentary retinopathy, a generalized reduction in muscle mass, a mild right hemiparesis, and a right homonymous hemianopsia. The right hemiparesis resolved, but the visual-field defect persisted. An electroretinogram was abnormal, consistent with early-stage pigmentary retinopathy. Clotting studies, electrocardigram, Westergren sedimentation rate, anti-nuclear antibodies, anti-DNA antibodies, cardiolipin antibodies, and protein C and protein S assays were negative. The hematology profile, creatinine phosphokinase, creatinine clearance, and blood chemistries were normal, except for a mildly decreased serum CO_2. Total thyroxine was 3.7 μg/mL (normal, 4.6 to 11.0) with a normal thyrotropin of 2.90 U/mL (normal, 0.50 to 4.80). Anti–islet cell antibodies were negative.

Fasting blood and 24-hour urine collection for amino acids demonstrated mild increases in blood alanine of 853 μmol/L (95% tolerance level, 635) and in urine alanine of 1,246 nmol/mg creatinine (95% tolerance level, 530), as well as in urine glycine of 8,856 nmol/mg creatinine (95% tolerance level, 2,687). Urine organic acids were normal, but blood lactate level was mildly elevated at 1,322 μmol/L (95% tolerance level, 1,118). The diagnosis of MELAS was confirmed by demonstrating the presence of the MTTL1*MELAS3243G mutation in leukocyte and platelet mtDNA.

OXPHOS Evaluation

A skeletal muscle biopsy was performed on subjects no. 1 and 2. In subject no. 1, muscle histochemistry was normal except for a single ragged-red fiber. Electron microscopy was normal. (For unknown reasons, the mtDNA rearrangement harbored by patient no. 1 and her maternal-lineage family members does not produce the ragged-red fiber myopathy frequently associated with mtDNA rearrangements and mitochondrial tRNA mutations.[3,4]) Subject no. 2 had normal histochemistry and electron microscopy.

OXPHOS enzymology was performed on skeletal muscle mitochondria isolated from subjects no. 1 and 2 (Table 1). In subject no. 1, complex III specific activity was decreased to less than the 5% tolerance level. The sonicated and freeze-thawed preparations used for complex IV analysis were near the 5% tolerance level. Complex I, I + III, and IV(FT) assays for subject no. 2 were at the 5% tolerance level.

FSIGT Studies

All three subjects demonstrated insulin sensitivity (SI) and glucose effectiveness (SG) in a range typical of individuals with NIDDM. As a basis for comparison, *z* scores were calculated based on mean values obtained from 11 normal controls from a previous study using the same technique.[14] Figure 1 shows endogenous insulin production and insulin sensitivity for the three study subjects (subject no. 2 studied on three separate occasions) compared with 11 normal subjects.

Subject no. 1 had well-established, poorly compensated diabetes, as indicated by a fasting glucose of 13.9 mmol/L. Diet was her only treatment at the time of study. First-phase insulin response was detectable but clearly abnormal (Table 2). There was little second-phase response. The insulin sensitivity index was $0.30 \times 10^{-4} \times \text{min}/\mu\text{U/mL}$, well below the predicted normal (z score $= -2.33, P < .01$). Glucose effectiveness was 0.022/min.

Table 1. Skeletal Muscle OXPHOS Enzymology

OXPHOS Assay	Normal Range (mean ± SD)	Patient No. 1	Patient No. 2
Complex I	106 ± 46	66	33*
	5% TL = 33		
Complex I + III	262 ± 93	157	103*
	5% TL = 105		
Complex II + III	526 ± 140	159*	348
	5% TL = 276		
Complex III	1,389 ± 368	686*	998
	5% TL = 733		
Complex IV (FT)	1,210 ± 353	654	576*
	5% TL = 589		
Complex IV (SON)	1,626 ± 407	963	1,290
	5% TL = 894		

Abbreviations: FT, freeze-thaw; SON, sonicated.
*At or <5% tolerance level.

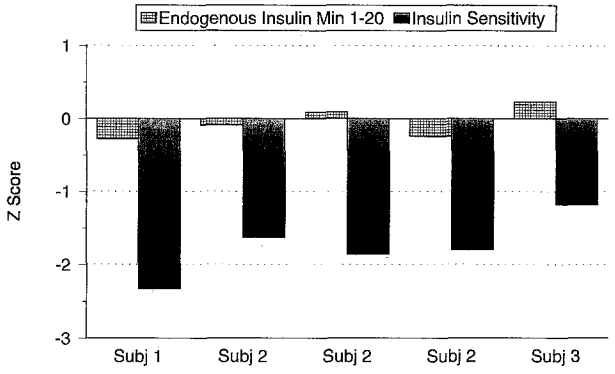

Fig 1. z scores for endogenous insulin release, measured as AUC (pmol/L/min) for 20 minutes following intravenous glucose challenge, and insulin sensitivity indices following FSIGT are shown for each study subject. Subject no. 2 was studied on 3 occasions over 16 months. z scores are based on 11 normal subjects studied using the same technique.

Subject no. 2 was studied on three separate occasions (Table 3). Throughout the 16-month interval of study, she was treated with glyburide. Her initial study was performed shortly after she was discovered to have diabetes. She reported an 8-kg weight loss before the diagnosis and was started on glyburide as initial therapy. During her study evaluation, she received diabetes education with recommendations of diet and exercise modification. On all three occasions, she continued her medication until the morning of study.

First-phase insulin release was poor at all three evaluations as determined by either peak insulin or AUC for minutes 1 to 10 after intravenous glucose, a response typical of subjects with NIDDM. Insulin AUC for minutes 1 to 20, which includes some second-phase response, is probably a better reflection of endogenous insulin release, because loss or blunting of first-phase insulin response with preservation of second-phase response is a characteristic of NIDDM. Even so, the endogenous insulin response to intravenous glucose was poor.

Compared with the initial study, subject no. 2 appeared better compensated when studied 10 months later. Fasting glucose levels were lower (7.3 *v* initial 8.4 mmol/L), fasting insulin was higher (237 *v* initial 97 pmol/L), and first-phase insulin release was increased fourfold (216 *v* initial 57 pmol/min). By 16 months after the initial study, fasting glucose returned to the initial value despite a fasting insulin level of 140% of the initial value, and first-phase insulin response to glucose was lower. Total endogenous insulin release (1 to 20 minutes before insulin injection) was much lower than in previous studies (AUC, 262 *v* 778 initial and 1,372 pmol/L × min at 10 months). Minimal-model estimation of insulin sensitivity was $1.23 \times 10^{-4} \times$ min/μU/mL at initial evaluation, typical of NIDDM. This value had decreased 25% 10 months later ($0.92 \times 10^{-4} \times$ min/μU/mL) and 20% below the initial value ($1.00 \times 10^{-4} \times$ min/μU/mL) 16 months later. All three insulin sensitivity values were less than normal (z score > -1.63, $P < .05$). Glucose effectiveness as calculated by the minimal model showed little change.

Table 2. Metabolic Characteristics of Subjects No. 1 and 3

Parameter	Subject No. 1	Z Score	Subject No. 3	Z Score
Weight (kg)	56.3	−0.74	31.3	−2.05*
Fasting glucose (mmol/L)	13.9	36.0*	4.4	−0.58
Fasting insulin (pmol/L)	79	0.41	36	−0.94
Peak insulin for minutes 1-10 (pmol/L)	127	−1.16	215	−1.02
First-phase insulin AUC (pmol/min)	93	N/A	1,235	N/A
Insulin 1- to 20-minute AUC (pmol/min)	119	−0.28	1,869	0.23
SI ($\times 10^{-4} \times$ min/pmol/L)	0.04	−2.33*	0.27	−1.18
SG (min^{-1})	0.022	−0.09	0.015	−0.73

NOTE. Z scores are based on mean values obtained from 11 normal controls previously reported using the same technique.

*$P \leq .01$.

Subject no. 3 had normal glucose tolerance at the time of study based on a standard criteria for a 2-hour, 75-g oral glucose tolerance test.[18] FSIGT responses are shown in Table 2. The first-phase insulin response was normal (AUC, 1,235 pmol/L × min), but insulin sensitivity was low ($1.86 \times 10^{4} \times$ min/μU/mL), within the 10th percentile of predicted levels based on previously studied normal subjects.[14] The value for glucose effectiveness was 0.016/min.

DISCUSSION

Diabetes mellitus is a common disease affecting as many as 5% to 10% of Japanese and Western populations.[19] Due to the high frequency of this disease in the population, identification of genes that increase an individual's risk of developing diabetes mellitus is important for developing an understanding of the disease pathogenesis and for optimizing patient management. In one subgroup of patients, development of diabetes mellitus is associated with mtDNA rearrangements[3,4,20] or mtDNA mutations[5,21] that impair OXPHOS function. Van den Ouweland et al[22] proposed the term, MIDD, for this subgroup. To date, the most common cause of MIDD is the MTTL1*MELAS3243G mutation.

In three large screening studies performed in Japan involving patients with NIDDM and IDDM[5,7,23] and in two European studies involving NIDDM patients,[22,24] the prevalence of the MTTL1*MELAS3243G mutation was similar, occurring in approximately 1% to 1.5% of individuals screened. In these studies, deafness appeared to be the most important clinical predictor for the presence of the MTTL1*MELAS3243G mutation, and was present in at least 65% of affected individuals.[5] The presence of diabetes mellitus in at least one maternal-lineage family member also was an important predictor for the MTTL1* MELAS3243G mutation. Although anti-islet cell antibodies have been reported in diabetic patients with the MTTL1*MELAS3243G mutation,[25] they are usually not found.[7,22,26]

Although most diabetic patients with the MTTL1* MELAS3243G mutation are characterized as having NIDDM at initial diagnosis, they ultimately require insulin treatment. In one large study, 55% (24 of 44) of the affected probands and maternal-lineage relatives were treated with insulin.[7] Katagiri et al[5] reported a duration of oral hypogly-

Table 3. Metabolic Characteristics of Subject No. 2 on Three Occasions Over 16 Months

Parameter	Initial Glyburide (5 mg)	Z Score	10 mo Later Glyburide (2.5 mg)	Z Score	16 mo Later Micronized Glyburide (6 mg)	Z Score
Weight (kg)	51.6	−0.98	61.8	−0.45	58.5	−0.62
Hemoglobin A_{1c} (%)*	8.1		6.2		9.0	
Fasting glucose (mmol/L)	8.4	15.0‡	7.3	10.6‡	8.4	15.0‡
Fasting insulin (pmol/L)	97	0.97	237	5.38†	136	2.20‡
Peak insulin in minutes 1-10 (pmol/L)	154	−1.10	287	−0.91	162	−1.11
First-phase insulin AUC (pmol/min)	57	N/A	216	N/A	64	N/A
Insulin 1- to 20-minute AUC (pmol/min)	778	−0.09	1,372	0.09	262	−0.24
SI ($\times 10^{-4}$ × min/pmol/L)	0.17	−1.63†	0.13	−1.86†	0.14	−1.80†
SG (min^{-1})	0.015	−0.73	0.013	−0.91	0.016	−0.64

NOTE. Z scores are based on mean values obtained from 11 normal controls using the same technique.[14]

*Reference range, 3.4% to 6.4%.

†$P \leq .05$.

‡$P \leq .01$.

cemic therapy in their subjects of less than 8 years.[5] To date, the incidence of complications such as cataracts, diabetic retinopathy, peripheral vascular disease, and neuropathies in these patients has not been assessed.

OXPHOS impairment could result in impairment of insulin release by at least two mechanisms. The key signaling event in insulin release is an increase in cytosolic ATP, which produces closure of ATP-dependent potassium channels in the β-cell membrane, allowing calcium influx and membrane depolarization. The mechanism by which glucose metabolism promotes ATP-potassium channel closure is controversial, but Dukes et al[27] proposed that NADH derived from glycolysis and converted to ATP by mitochondria is central to the signaling process. An alternate mechanism by which impairment in cytosolic OXPHOS could result in impaired carbohydrate metabolism is via impairment in the function of ATP-dependent hexokinase, which catalyzes intracellular conversion of glucose to glucose-6-phosphate, a rate-limiting step in glucose metabolism. Enzyme activity is influenced by binding to porin, an outer mitochondrial membrane channel. Four isoforms of hexokinase have been described. Hexokinase IV (glucokinase) found in β cells has a K_m of approximately 10 mmol/L, so that the rate of glucose phosphorylation by glucokinase is affected by physiologic changes in blood glucose concentration and is important for regulating pancreatic β-cell insulin release. Point mutations in the glucokinase gene have been shown to result in a form of diabetes known as maturity-onset diabetes in the young.[28,29]

Whereas glucose-stimulated insulin secretion in diabetic subjects with OXPHOS diseases is impaired,[5,7,23,26] our study suggests that insulin action is also impaired and may represent an earlier defect. Insulin resistance was demonstrated in a single patient with the MTTL1*MELAS3243G mutation by Kanamori et al[30]; however, others have reported normal insulin sensitivity in affected individuals.[7,31] Variability in glucose homeostasis observed in patients with mtDNA mutations may result, in part, from the random segregation of mutant and normal mitochondria during cell division, so that the proportion of mitochondria with mutant mtDNA shows marked variation between tissues even in the same individual.[1] Studying subject no. 2 over a 16-month course of observation has demonstrated persistent insulin resistance with an initial compensatory increase in insulin levels, followed by a subsequent diminution of insulin release as hyperglycemia increased, the classic horseshoe pattern described by DeFronzo[32] and others.

Study subject no. 3, at the age of 17, had not developed diabetes but had the mtDNA mutation, deafness, and a mother with MIDD. He had no demonstrable defect in insulin secretion, but insulin sensitivity was at the low range of predicted values for normal. The normal range in minimal-model calculations of insulin sensitivity has not been clearly defined. Wide variations in insulin sensitivity levels have been reported in subjects with normal glucose tolerance. Contributing to the variation have been differences in FSIGT techniques among investigators. Intravenous glucose has been given alone, with tolbutamide, or with insulin. Using the insulin-modified FSIGT technique, Taniguchi et al[33] reported a mean insulin sensitivity of $1.13 \pm 0.15 \times 10^{-4}$ min/pmol/L (n = 15 normal subjects). Kahn et al[34] analyzed FSIGT results from 93 subjects who had served as normal controls from 1983 to 1987 and found a mean insulin sensitivity of $0.71 \pm 0.47 \times 10^{-4}$ min/pmol/L. The value of 0.27×10^{-4} min/pmol/L in subject no. 3 is lower than in normal subjects we have studied,[14] but is within the 10th percentile given the variation in values.

We would like to speculate that decreased activity of hexokinase II, the hexokinase isoform expressed in muscle, could account for the insulin resistance we observed in our subjects. Reduced expression of hexokinase II has been associated with insulin resistance in animal models[35] and with myopathy and increased lactate production in an individual case report.[36] Our observations suggest that insulin resistance is present early in MIDD. As demand for insulin exceeds the secretory capacity of the β cell, hyperglycemia results. Impairment in ATP-dependent hexokinase activity could account for both defects in insulin secretion and insulin action described in this syndrome.

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