Impaired Mitochondrial Function and Insulin Resistance of Skeletal Muscle in Mitochondrial Diabetes

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OBJECTIVE — Impaired muscular mitochondrial function is related to common insulin resistance in type 2 diabetes. Mitochondrial diseases frequently lead to diabetes, which is mostly attributed to defective β-cell mitochondria and secretion.

RESEARCH DESIGN AND METHODS — We assessed muscular mitochondrial function and lipid deposition in liver (hepatocellular lipids [HCLs]) and muscle (intramyocellular lipids [IMCLs]) using $^{31}P/^{1}H$ magnetic resonance spectroscopy and insulin sensitivity and endogenous glucose production (EGP) using hyperinsulinemic-euglycemic clamps combined with isotopic tracer dilution in one female patient suffering from MELAS (myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) syndrome and in six control subjects.

RESULTS — The MELAS patient showed impaired insulin sensitivity (4.3 vs. 8.6 ± 0.5 mg · kg^{-1} · min^{-1}) and suppression of EGP (69 vs. 94 ± 1%), and her baseline and insulin-stimulated ATP synthesis were reduced (7.3 and 8.9 vs. 10.6 ± 1.0 and 12.8 ± 1.3 μmol · l^{-1} · min^{-1}) compared with those of the control subjects. HCLs and IMCLs were comparable between the MELAS patient and control subjects.

CONCLUSIONS — Impairment of muscle mitochondrial fitness promotes insulin resistance and could thereby contribute to the development of diabetes in some patients with the MELAS syndrome.

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The MELAS (myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) syndrome is caused by a maternally inherited mtDNA mutation, resulting in defective cellular respiration. MELAS-associated diabetes has been primarily attributed to insufficient insulin secretion due to mitochondrial dysfunction of pancreatic β-cells. Insulin-resistant populations show reduced mitochondrial function and increased soleus muscle lipids (intramyocellular lipids [IMCLs]) and liver lipids (hepatocellular lipids [HCLs]) (1). The sequence of events, particularly the possibility of muscle mitochondria leading to insulin resistance, is unclear.

RESEARCH DESIGN AND METHODS

— One female patient (age 37 years, BMI 24 kg/m^2, A1C 7.4%) suffering from MELAS (mtDNA mutation A3243G, ~60% heteroplasmy in leukocytes, confirmed by PCR amplification) was compared with six nondiabetic female control subjects (age 45 ± 4 years, BMI 24 ± 1 kg/m^2, A1C 5.2 ± 0.1%). The protocol was approved by an institutional ethics board, and written informed consent was obtained. The patient developed bilateral labyrinthine hypacusis and insulin-dependent diabetes at 26 years of age, underwent surgery for ptosis, and showed vascular lesions in her periventricular white matter.

Whole-body insulin sensitivity (insulin-mediated glucose disposal, *M*) and endogenous glucose production (EGP) were assessed during a normoglycemic-hyperinsulinemic clamp with infusion of insulin (40 mU · m body surface area^{-1} · min^{-1}) (Actrapid; Novo Nordisk, Copenhagen, Denmark) and 20% dextrose containing 2%-enriched D-[6,6-$^{2}H_2$]glucose (2).

Plasma glucose was measured by the glucose oxidase method (Glucose analyzer II; Beckman Coulter, Brea, CA). Free fatty acid (FFA) was measured microfluorimetrically (Wako Chemicals, Richmond, VA). Insulin was measured by radioimmunoassay (2).

Open-air spirometry (MasterScreen CPX; Jaeger Viasys Healthcare, Hoechberg, Germany) was combined with continuous heart rate recording (SporttesterPE4000; Polar Electro, Oulu, Finland). Resting energy expenditure (REE) was assessed using the Weir equation: REE = [3.9(V_{O_2}) + 1.1(V_{CO_2})]1.44. Exercise testing was performed on an electronically braked cycle ergometer (Lode-Excalibur Sport, Groningen, the Netherlands).

$^{31}P/^{1}H$ magnetic resonance spectroscopy (MRS) was performed at baseline and repeated between 120 and 240 min of the clamp to assess flux through ATP synthase (fATPase) in gastrocnemius muscle with saturation transfer experiment (3-T spectrometer; Bruker, Ettlingen, Germany) (2). Glucose-6-phosphate (G-6-P) and inorganic phosphate (P_i) were measured from the ratio of integrated respective peak intensities and β-ATP resonance intensity (2). Localized ^{31}P-MRS was performed to assess PCr recovery using STEAM (the stimulated echo acquisition

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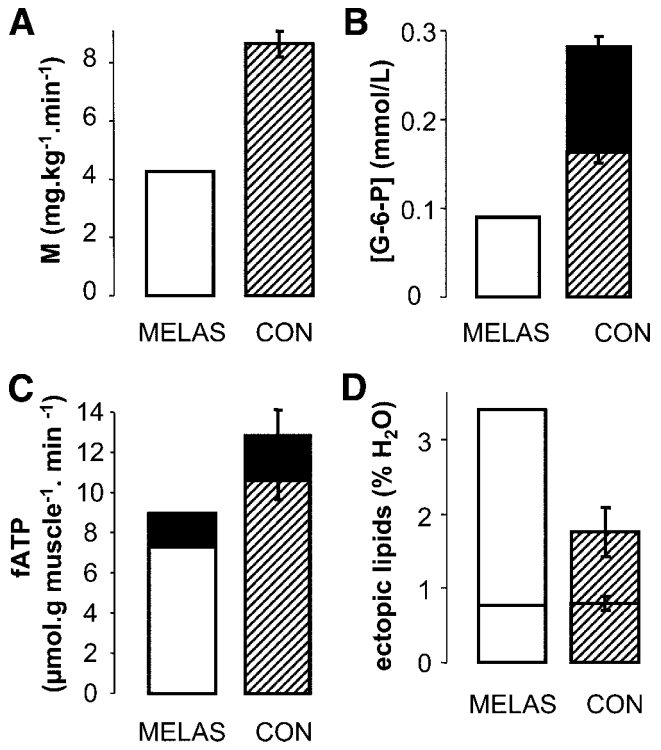

Figure 1—*Whole body insulin sensitivity (*M *value) (*A*), glucose phosphorylation/transport (IMCL G-6-P) (*B*), and IMCL ATP synthesis as well as ectopic lipids in skeletal muscle (IMCL, lower portion of the columns) (*C*) and liver (HCL, total columns) (*D*) in a female patient with MELAS syndrome (□) compared with matched control subjects (CON, ▨,* n = *6).* ■*, Insulin-stimulated increases in G-6-P and ATP synthesis.*

mode) and the time domain fit routine AMARES (advanced method for accurate, robust, and efficient spectral fitting of MRS data). Aerobic plantar flexion exercise was performed at 50% maximal contraction until fatigue. PCr amplitudes were fitted to a mono-exponential curve using nonlinear least squares. Ectopic lipids were measured with ^{1}H-MRS (2). The patient's PCr recovery was compared with that of healthy male subjects from a previous study of ours (3), and REE was compared with the value predicted by the Harris-Benedict equation for women: (665.1 + 9.6 × weight) + (1.8 × height) − (4.7 × age). All other tests were performed on the patient and six control subjects, and data are shown as means ± SEM.

RESULTS

Glucose and FFA metabolism

The MELAS patient had normal fasting EGP (1.7 vs. 1.7 ± 0.1 mg · kg^{-1} · min^{-1}), plasma FFA (385 µmol/l), and insulin suppression of lipolysis (95%) in comparison with the control subjects. EGP suppression (69 vs. 94 ± 1%) and *M* (4.3 vs. 8.6 ± 0.5 mg · kg^{-1} · min^{-1}) were markedly lower in the patient than in the control subjects (Fig. 1*A*).

Energy expenditure

The patient's respiratory quotient was 0.88, indicating reliance on glucose oxidation during fasting. REE was lower than predicted (1,108 vs. 1,355 kcal/24 h). Maximal power (88 W) and $V\mathrm{O}_{2max}$ (19.4 mg · kg^{-1} · min^{-1}) were reduced.

Intracellular metabolites and ATP synthesis

G-6-P did not increase after insulin stimulation, proving impaired muscle glucose transport/phosphorylation (control: +75% vs. baseline, $P < 0.001$) (Fig. 1*B*). Fasting and insulin-stimulated fATPase were lower in the MELAS patient (7.3 and 8.9 µmol · l^{-1} · min^{-1}) than in the control subjects (10.6 ± 1.0 vs. 12.8 ± 1.3 µmol · l^{-1} · min^{-1}, $P < 0.05$) (Fig. 1*C*). The patient performed plantar flexion for 6.4 min, resulting in ~60% PCr depletion, demonstrating exhaustive exercising. Postexercise PCr recovery was twice (66 ± 17 s) that of healthy volunteers (3), indicating compromised mitochondrial fitness (4). Resting ATP concentration (4.7 mmol/l) and PCr-to-P_i ratio (5.0) were reduced by ~40% compared with those in healthy volunteers studied under identical conditions (5). HCLs, albeit within the normal range (<5%) (6), were higher in the MELAS patient than in the control subjects, whereas IMCLs were similar (Fig. 1*D*).

CONCLUSIONS

— The MELAS patient exhibited severe reductions in ATP concentrations and synthesis at baseline, after exercise, and during insulin stimulation. This was paralleled by muscular and hepatic insulin resistance. While the cause of insulin resistance could likely be explained by primary defects of mitochondrial number and fitness, both abnormalities could also result from lipotoxicity. Lipotoxicity can cause not only β-cell dysfunction but also muscular insulin resistance via inflammatory pathways, decreasing fat oxidation and raising IMCLs (7). Normal fasting and insulin suppression of FFA as well as low IMCLs would argue against the operation of lipotoxicity in our patient. However, her severe hepatic insulin resistance and higher HCLs are in line with the contention that hepatic signals, e.g., release of lipids (6), contributed to muscular insulin resistance and mitochondrial dysfunction. Finally, despite good long-term metabolic control, chronic hyperglycemia could have aggravated insulin resistance and mitochondrial dysfunction via glucotoxicity.

Similar to most MELAS patients, our patient had normal body mass, manifested diabetes in early adulthood, and required insulin treatment (8). Although some studies found that patients with mitochondrial diabetes are insulin resistant (8), others reported normal insulin sensitivity, suggesting that the A3243G mutation in skeletal muscle does not play a causative role in diabetes development (9). As the majority of studies show that MELAS patients present with impaired glucose-stimulated insulin secretion, occurrence of diabetes has been attributed to impaired β-cell function, which strongly depends on intact mitochondrial metabolism (7). Our patient presented with severe insulin resistance, impaired insulin-stimulated glucose transport/phosphorylation, reduction of myocellular concentrations, and synthetic flux of ATP. These alterations are similar to findings in insulin-resistant populations (elderly, obese nondiabetic humans, and first-degree relatives of patients with type 2 diabetes) who are at increased risk of diabetes (10,11). Previous reports found

increased prevalence of A3243G in patients with type 2 diabetes (12). Thus, insulin resistance along with lower muscular mitochondrial fitness could contribute to the manifestation of diabetes in MELAS patients.

In conclusion, impairment of muscle mitochondrial fitness contributes to insulin resistance in our patient with MELAS and diabetes, which resembles the mechanism described for first-degree relatives of type 2 diabetic patients. However, given the broad spectrum of MELAS defects and heterogeneity of insulin sensitivity in these patients, the present report does not permit the conclusion that MELAS is a typical model for the pathogenesis of type 2 diabetes.

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