Impaired mitochondrial function and insulin resistance of skeletal muscle in mitochondrial diabetes

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Objective: Impaired muscular mitochondrial function relates to common insulin resistance in type 2 diabetes. Mitochondrial diseases frequently lead to diabetes, which is mostly attributed to defective ß-cell mitochondria and secretion.

Research Design and Methods: We assessed muscular mitochondrial function and lipid deposition in liver (HCL) and muscle (IMCL) using $^{31}$P/$^1$H magnetic resonance spectroscopy (MRS), insulin sensitivity and endogenous glucose production (EGP) by hyperinsulinemic-euglycemic clamps combined with isotopic tracer dilution in one female suffering from the MELAS syndrome and in six controls (CON).

Results: The patient showed impaired insulin sensitivity (4.3 vs. CON: 8.6±0.5 mg.kg$^{-1}$.min$^{-1}$) and suppression of EGP (69% vs. 94±1%). Baseline and insulin-stimulated ATP synthesis were reduced (7.3 and 8.9 µmol.l$^{-1}$.min$^{-1}$ vs. 10.6±1.0 and 12.8±1.3 µmol.l$^{-1}$.min$^{-1}$). HCL and IMCL were comparable with CON.

Conclusions: Impairment of muscle mitochondrial fitness promotes insulin resistance and could thereby contribute to the development of diabetes in certain patients with the MELAS syndrome.

Abbreviations
CON Control subjects
EGP Endogenous glucose production
fATPase Flux through ATP synthase
FFA Free fatty acids
G-6-P Glucose-6-phosphate
HCL Accumulation of hepatocellular lipids
IMCL Accumulation of intramyocellular lipids
mtDNA Mitochondrial DNA
MELAS syndrome Myopathy, Encephalopathy, Lactate acidosis and Stroke-like episodes
PCr Phosphocreatine
REE Resting energy expenditure
Insulin resistance in MELAS

The MELAS syndrome (myopathy, encephalopathy, lactic acidosis, stroke-like episodes) is caused by a maternally-inherited mitochondrial DNA (mtDNA) mutation, resulting in defective cellular respiration. MELAS-associated diabetes mellitus 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 and liver lipids (IMCL, HCL) (1). The sequence of events, particularly a possible role of muscle mitochondria leading to insulin resistance, is unclear.

HISTORY AND EXAMINATION

One female (age: 37 years, body mass index: 24 kg/m², HbA1c: 7.4%) suffering from MELAS (mtDNA mutation A3243G, ~60% heteroplasmy in leukocytes, confirmed by PCR-amplification) was compared with six nondiabetic females (CON: 45±4 years, BMI: 24±1 kg/m², HbA1c: 5.2±0.1%). The protocol was approved by our institutional ethics board, and written informed consent was obtained. The patient developed bilateral labyrinthine hypacusis and insulin-dependent diabetes mellitus at 26 years, underwent surgery of ptosis and showed vascular lesions in 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)⁻².min⁻¹; Actrapid, Novo, Denmark] and 20% dextrose containing 2-%-enriched D-[6,6-²H₂]glucose (2).

Plasma glucose was measured by the glucose oxidase method (Glucose analyzer II, Beckman, CA), FFA microfluorimetrically (Wako Chem USA Inc., VA), insulin by radioimmunoassay (2).

Open-air spirometry (Jaeger/Viasys MasterScreen CPX, Germany) was combined with continuous heart-rate recording (SporttesterPE4000, Polar Electro, Finland). Resting energy expenditure (REE) was assessed using the Weir-equation:

\[ \text{REE} = [3.9(\text{VO}_2) + 1.1(\text{VCO}_2)]^{1.44}. \]

Exercise testing was performed on an electronically-braked cycle ergometer (Lode-Excalibur Sport, Netherlands).

³¹P MRS was performed at baseline and repeated between 120 min and 240 min of the clamp to assess flux through ATP synthase (fATPase) in gastrocnemius muscle with the saturation transfer experiment (3-T spectrometer, Bruker, 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 ³¹P MRS was performed to assess PCr-recovery using STEAM (stimulated echo acquisition mode) and 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 fit routine. Ectopic lipids were measured with ¹H MRS (2). The patient’s PCr recovery was compared to that of healthy male humans from our previous study (3), REE was compared to the value predicted by the Harris-Benedict equation for women:
(665.1+9.6xweight)+(1.8xheight)-
(4.7xage). All other tests were performed
in the patient and the six CON
(mean±SEM).

INVESTIGATION

Glucose and FFA metabolism:
The patient had normal fasting EGP (1.7
vs. CON: 1.7±0.1 mg.kg⁻¹.min⁻¹), plasma
FFA (385 µmol/l), and insulin-suppression
of lipolysis (95%). EGP suppression (69%
v. 94±1%) and M were markedly lower
(4.3 vs. 8.6±0.5 mg.kg⁻¹.min⁻¹, Figure 1A).

Energy expenditure: The
respiratory quotient was 0.88 indicating
reliance on glucose oxidation during
fasting. REE was lower than predicted
(1108 vs. 1355 kcal/24 h). Maximal power
(88 W) and VO₂max (19.4 ml.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
(CON: +75% vs. baseline, p<0.001,
Figure 1B).

Fasting and insulin-stimulated
fATPase were lower (7.3, 8.9 µmol.l⁻¹.min⁻¹)
than in CON (10.6±1.0 vs. 12.8±1.3
µmol.l⁻¹.min⁻¹, p<0.05, Figure 1C). The
patient performed plantar flexion for 6.4
min resulting in ~60% PCR-depletion
demonstrating exhaustive exercising.
Post-exercise 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-Pi ratio (5.0)
were reduced by ~40% compared to
healthy volunteers studied under identical
conditions (5). HCL, albeit within the
normal range (<5%) (6), were higher
than, whereas IMCL were similar in CON
(Figure 1D).

CONCLUSIONS

The MELAS patient exhibited
severe reduction of 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
not only cause β-cell dysfunction but also
muscular insulin resistance via
inflammatory pathways decreasing fat
oxidation and raising IMCL (7). Normal
fasting and insulin-suppression of FFA as
well as low IMCL would argue against the
operation of lipotoxicity in our patient.
However, her severe hepatic insulin
resistance and higher HCL 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 female had normal body mass,
manifested with 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 does not play a
causative role for 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, 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 T2DM.

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Figure 1
Whole body insulin sensitivity (M value) (A), glucose phosphorylation/transport, (intramyocellular glucose-6-phosphate) (B), intramyocellular ATP synthesis (C) as well as ectopic lipids in skeletal muscle (IMCL, bottom of the columns) and liver (HCL, total columns) (D) in a female patient with MELAS syndrome (empty columns) compared to matched controls (CON: n=6, hatched columns). Insulin-stimulated increases in glucose-6-phosphate and ATP synthesis are given as black columns.