Diminished Insulin Secretory Response to Glucose but Normal Insulin and Glucagon Secretory Responses to Arginine in a Family With Maternally Inherited Diabetes and Deafness Caused by Mitochondrial tRNA$^{LEU(UUR)}$ Gene Mutation

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OBJECTIVE — The effects of glucose, arginine, and glucagon on β-cell function as well as α-cell response to arginine were studied in a family with mitochondrial diabetes.

RESEARCH DESIGN AND METHODS — The function of α- and β-cells was assessed in all five siblings carrying the mitochondrial tRNA Leu(UUR) gene mutation at position 3243 and compared with six sex-, age-, and weight-matched control subjects. Insulin and C-peptide responses were evaluated by intravenous glucagon application, intravenous arginine stimulation test, and intravenous glucose tolerance test. Glucagon secretion was assessed during the arginine stimulation test.

RESULTS — The glucose disappearance constant (K_g) value (mean ± SEM 0.61 ± 0.04 vs. 1.1 ± 0.04, $P = 0.0002$) as well as the acute insulin response to glucose (area under the curve [AUC] 0–10 min, 77.7 ± 50.7 vs. 1,352.3 ± 191.5 pmol/l, $P = 0.0004$) were decreased in all patients. Similarly, glucagon-stimulated C-peptide response was also impaired (728 ± 111.4 vs. 1,526.7 ± 157.7 pmol/l, $P = 0.005$), whereas the insulin response to arginine (AUC) was normal (1,346.9 ± 710.8 vs. 1,083.2 ± 132.5 pmol/l, $P = 0.699$). Acute glucagon response to arginine (AUC) was normal but tended to be higher in the patients than in the control subjects (181.7 ± 47.5 vs. 90.0 ± 21.1 pmol/l, $P = 0.099$).

CONCLUSIONS — This study shows impaired insulin and C-peptide secretion in response to a glucose challenge and to glucagon stimulation in diabetic patients with mitochondrial tRNA Leu(UUR) gene mutation, although insulin and glucagon secretory responses to arginine were normal.

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Abbreviations: AIR_g, acute insulin response to glucose; AUC, area under the curve; CV, coefficient of variation; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; K_g, glucose disappearance constant; MIDD, maternally inherited diabetes and deafness.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The triad of diabetes, maternal inheritance, and impaired hearing is also known as maternally inherited diabetes and deafness (MIDD) (1,2). In most cases, this diabetes subtype is linked to an A-to-G transition at nucleotide position 3243 of the mitochondrial tRNA$^{LEU(UUR)}$ gene (3–9). MIDD is responsible for 0.5–1.5% of all cases of diabetes in the general population (2,10,11).

The pathophysiological mechanisms leading to mitochondrial diabetes are complex and may involve impaired insulin secretion, glucose toxicity, β-cell loss, and insulin resistance (12–16). Because mitochondrial oxidative phosphorylation plays a major role in glucose-induced insulin secretion (17), impairment of the insulin response to a glucose challenge is an early event and represents the major factor in the pathogenesis of hyperglycemia in mitochondrial diabetes (12,18,19). In contrast, the insulin response to stimuli other than glucose, such as glucagon and arginine, is preserved or only mildly impaired (12,13,18). It is unclear whether the mitochondrial DNA mutation also affects α-cell function.

The objective of the present study was to assess the effects of glucose, arginine, and glucagon on β-cell function as well as on the α-cell response to arginine in all siblings of a family with mitochondrial diabetes.

RESEARCH DESIGN AND METHODS

— Five siblings from a family with the mitochondrial tRNA$^{LEU(UUR)}$ point mutation of mitochondrial DNA were studied. The 3243 A-to-G transition in the mitochondrial tRNA$^{LEU(UUR)}$ gene was detected in DNA from peripheral lymphocytes using poly-

Table 1—*Anthropometric data and glucose metabolism*

Anthropometric data	MD1	MD2	MD3	MD4	MD5	Patients	Control subjects
Sex (M/F)	F	M	F	F	F	—	F/1M
Age (years)	28	38	35	36	33	34.0 ± 1.7	32.5 ± 1.8
BMI (kg/m^2)	21.8	23.9	18.9	21	24.7	22.1 ± 1	21.5 ± 0.9
Glucose metabolism	diabetes	diabetes	diabetes	IGT	diabetes	—	Normal
Age at diagnosis (years)	21	33	30	34	29	29.4 ± 5.1	—
Treatment	diet	insulin	insulin	diet	insulin	—	None
HbA_{1c} (%)	7.0	7.7	5.8	5.9	5.7	6.4 ± 0.4	4.6 ± 0.2
Fasting glucose (mmol/l)	8.0	4.1	7.4	4.5	6.7	6.1 ± 0.8	4.5 ± 0.1
Fasting glucagon (pmol/l)	10.2	22.8	13.7	15.3	24.7	17.3 ± 2.8	18.0 ± 3.6
Fasting insulin (pmol/l)	37	111	52	110	68	75.6 ± 15.1	65.0 ± 6.2
Fasting C-peptide (pmol/l)	440	270	370	630	770	496 ± 90	559 ± 52

Data are *n* or means ± SD unless otherwise indicated.

merase chain reaction amplification, *Apa*I digestion, acrylamide gel electrophoresis, and silver-nitrate staining (20). Metabolic studies were performed in all family members with the mitochondrial gene mutation. All subjects provided written informed consent. One subject, aged 36 years, had impaired glucose tolerance (IGT), whereas all others (28–38 years of age) had diabetes. Three subjects were treated with insulin and two were treated with diet only. The diabetes or IGT were diagnosed at 21–34 years of age. All subjects were of normal weight (mean ± SEM), with BMI 22.1 ± 1 kg/m^2 (Table 1), and none had diabetic late complications. Audiometric testing showed mild sensorineural hearing loss in three subjects. Echocardiography was normal in all patients, and ophthalmic examination with fundoscopy revealed no pigmentary retinopathy (Fig. 1).

A total of six healthy individuals with no family history of diabetes who were matched for age, weight, and sex were used as the control group. The patients had slightly elevated HbA_{1c} and fasting plasma glucose levels compared with the control subjects ($P = 0.006$ and $P = 0.047$, respectively) (Table 1). Fasting insulin, C-peptide, and glucagon levels did not differ significantly between mutation-positive subjects and control subjects (Table 1). Results of analyses for islet cell antibodies and antibodies to GAD 65, insulinoma-associated antigen 2, and insulin were negative in all patients.

Study design

Subjects and control subjects were studied in the outpatient clinic of the Division of Endocrinology and Diabetes at the University Hospital of Zürich. Insulin secretion was evaluated by intravenous glucose tolerance test (IVGTT), arginine stimula-

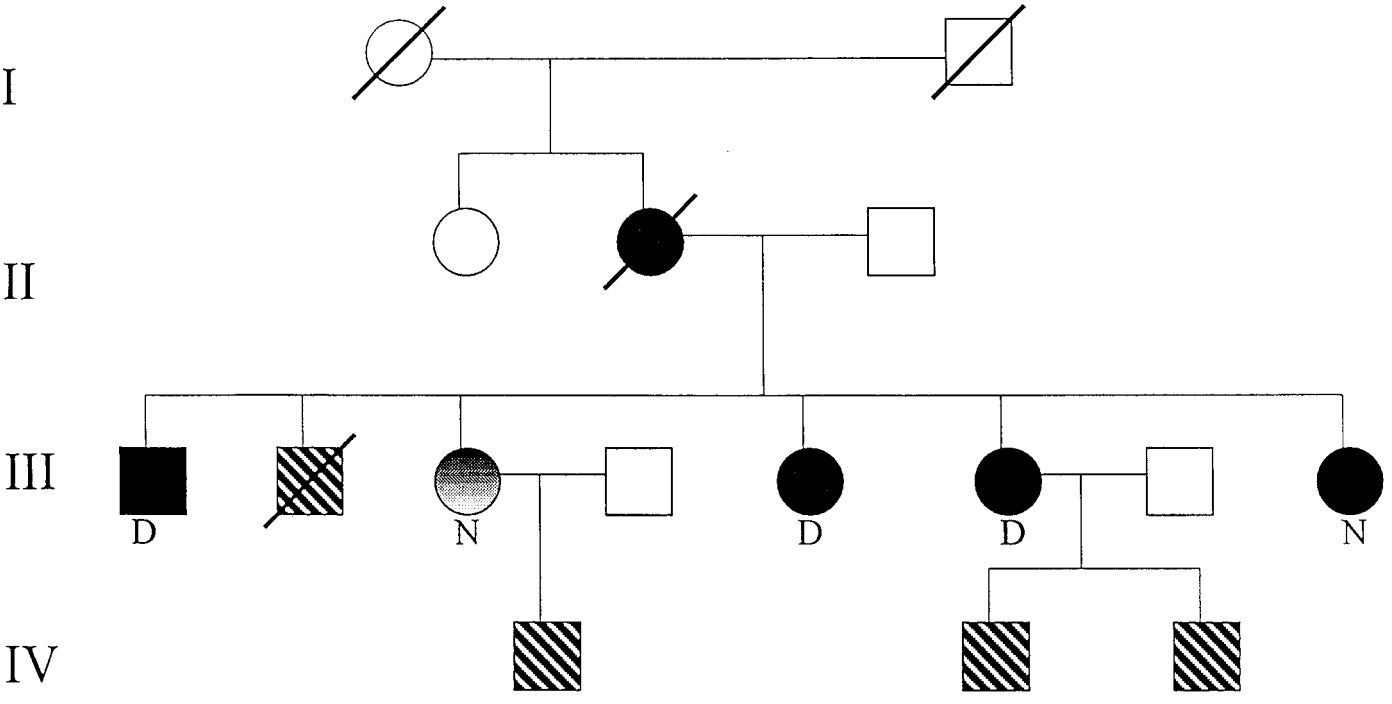

Figure 1—*Pedigree of the family with the mitochondrial tRNA $^{LEU(UUR)}$ gene mutation at base pair 3243. □, male; ○, female; ●, mutation with diabetes; ◍, mutation with IGT; ○, wild gene; ▨, untested; ∅, dead. D, sensorineural deafness; N, normal audiometric testing.*

Table 2—*β- and α-cell stimulation tests*

	Subjects	Control subjects	*P*
n	5	6	—
C-peptide to glucagon (mean 6′ and 10′, pmol/l)	728 ± 111.4	1,526.7 ± 157.7	0.005
Insulin to arginine (AUC 0–10, pmol/l × min)	1,346.9 ± 710.8	1,083.2 ± 132.5	0.699
Insulin to glucose (AUC 0–10, pmol/l × min)	77.7 ± 50.7	1,352.3 ± 191.5	0.0004
Glucagon to arginine (AUC 0–10, pmol/l)	181.7 ± 47.5	90.0 ± 21.1	0.099

Data are *n* or means ± SD.

tion test, and intravenous glucagon application. Glucagon secretion was assessed by arginine stimulation test. Studies were started at 0800–0900 with subjects in recumbent position after an overnight fast of 10–12 h; glucagon stimulation test and stimulations with arginine and glucose were performed on alternate days. Three patients were being treated with insulin. The last dose of short-acting insulin (Actrapid; Novo Nordisk, Copenhagen) was administered 12 h before the tests were started.

Glucagon test. Baseline levels of insulin, C-peptide, and plasma glucose were obtained. At time 0 min, 1 mg glucagon was injected intravenously, and blood samples for insulin, C-peptide, and plasma glucose were collected at 6 and 10 min (21).

Arginine stimulation test and IVGTT. Blood samples for measurement of basal glucose, insulin, C-peptide, and glucagon concentrations were collected at −10, −5, and 0 min. Arginine (5 g) in the form of L-arginine hydrochloride 21% was infused intravenously over 30 s (B. Braun Medical AG, Emmenbrücke, Switzerland). Samples were obtained at 2, 3, 4, 5, 7, 10, 15, 20, 25, and 30 min (22,23). After the arginine stimulation test, a 30-min washout period was allowed for prevention of carryover effects before the IVGTT. Baseline blood samples for insulin, C-peptide, and plasma glucose were collected, and 0.5 g glucose per kilogram body weight in a 50% glucose solution was injected intravenously over 30 s. Blood samples were collected at 1, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, and 60 min postinjection.

Assay procedures

All blood samples were collected in glass tubes with no additives and glass tubes containing 0.05 ml 15% fluid EDTA and 0.2 ml Trasylol (500 K.I.U. aprotinin/ml blood). Samples were placed on ice and centrifuged within 10 min. Plasma was then stored at −80°C until assayed. Plasma glucose was measured immediately by the glucose oxidase technique (Beckman Analyzer; Beckman, Fullerton, CA). Immunoreactive insulin was measured by solid-phase radioimmunoassay (intra-assay coefficient of variation [CV] 5%, interassay CV 4.9%) (Coat-A-Count Insulin; DPC, Los Angeles, CA). Measurement of C-peptide was performed with a solid-phase, chemiluminescent enzyme immunoassay (intra-assay CV 6.3%, interassay CV 6.3%) (Immulite C-peptide; DPC), and glucagon was measured by a double-antibody radioimmunoassay (intra-assay CV 4.1%, interassay CV 5.7%) (DPC).

Data analysis

Baseline fasting plasma glucose, insulin, and glucagon levels were calculated as the mean of the values recorded for three blood samples collected before the glucose injection. The glucose disappearance constant (K_g) was calculated from the IVGTT as the slope of the decline of the $\log_e$ (ln) plasma glucose between 10 and 30 min after the glucose injection multiplied by 100. The acute insulin response to glucose (AIR_g) and to arginine was calculated as the incremental area under the insulin curve between 0–10 min after the intravenous injection of glucose and between 0–10 min after injection of arginine, respectively. The glucagon secretory response to arginine was calculated as the incremental area under the glucagon curve between 0–10 min after arginine injection. The areas under the curves ($AUC_{Insulin}$ and $AUC_{Glucagon}$) were calculated by means of the trapezoidal rule with subtraction of the basal values. Results are given as means ± SEM. Data were analyzed with Statistica for Windows software (Statsoft, Tulsa, OK). Relations among variables of interest were analyzed pairwise (before and after intervention) with the paired Student's *t* test for dependent samples or with the Wilcoxon matched-pairs test, where appropriate. $P < 0.05$ was considered statistically significant.

RESULTS — The increase of plasma glucose on intravenous glucose bolus (IVGTT) was not different in the patients compared with the control subjects ($P = 0.16$). The K_g value was significantly lower in subjects with the mutation than in the control subjects (0.61 vs. 1.1; $P = 0.0002$), as an overall indicator of insulin secretion and insulin resistance.

β-Cell function: effects of glucagon, arginine, and glucose on insulin and C-peptide secretion

C-peptide concentrations at 6 and 10 min after intravenous application of 1 mg glucagon were subnormal in the patients and differed significantly from the control subjects ($P = 0.005$) (Table 2).

The first-phase insulin secretion upon intravenous glucose bolus (IVGTT), as shown in Fig. 2, was clearly impaired in all patients, and the calculated incremental $AUC_{Insulin}$ (0–10 min) was 77.7 ± 50.7 pmol/l × min (range −23 to 265 pmol/l × min) in affected subjects vs. 1,352.3 ± 191.5 pmol/l × min (532–2,106) in control subjects ($P = 0.0004$) (Table 2).

In contrast, the acute insulin response to arginine did not differ between the two groups (Fig. 3, Table 2).

α-Cell function: effects of arginine on glucagon secretion

Fasting plasma glucagon concentrations were not significantly different in patients compared with control subjects. The glucagon response to arginine, as demonstrated in Fig. 4, did not differ significantly between the patients and the control subjects but tended to be higher in the patient group. $AUC_{Glucagon}$ was 181.7 ± 47.5 vs. 90.0 ± 21.1 pmol/l × min in the affected subjects and the control subjects, respectively ($P = 0.099$).

CONCLUSIONS — All subjects with mitochondrial $tRNA^{LEU(UUR)}$ gene muta-

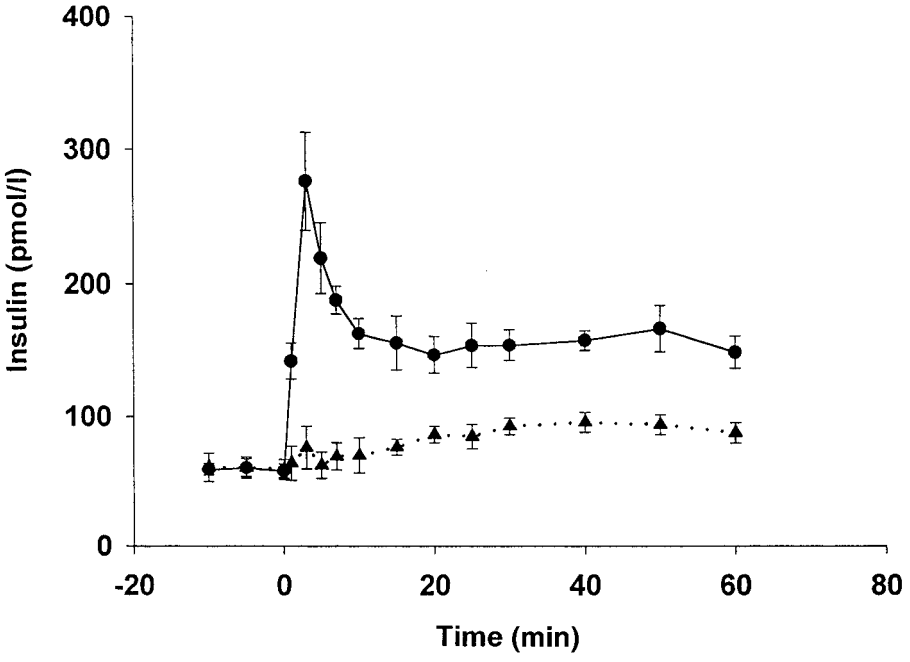

Figure 2— *Insulin response to intravenous glucose (IVGTT). ▲, patients; ●, controls.*

tion displayed a blunted early insulin response to glucose during IVGTT. These findings support the assumption that impaired insulin secretion to glucose stimulation is an early and crucial element in the development of hyperglycemia in mitochondrial diabetes, as previously reported (12,14,18). The C-peptide response to glucagon was not abolished but was impaired in all patients compared with normal subjects. Despite a different signaling pathway, an impaired insulin response on stimulation with glucagon seems to occur early in the disease process as well. Our results are in keeping with data from other studies (14,16) but at variance with the findings from Suzuki et al. (18), who reported that 12 patients with mitochondrial diabetes showed a normal increase in C-peptide after glucagon stimulation. Their patients had an older age of onset of diabetes (44 ± 10 years) compared with our subjects with MIDD, suggesting a less deleterious effect of the mitochondrial DNA mutation on the β-cell in their population. C-peptide response to glucagon, therefore, seems to be decreased, but not completely abolished, in most cases and normal in some cases.

The insulin response to arginine was preserved in our patients, which is consistent with the findings of Velho et al. (12). Several mechanisms for arginine-induced β-cell stimulation have been proposed. Direct depolarization of the plasma membrane caused by intracellular accumulation of the positively charged arginine might be responsible, at least in part, for the insulinotropic action of arginine (24–26). This mechanism of stimulation of insulin secretion by arginine may not involve changes in mitochondrial oxidative phosphorylation and intracellular production of ATP. Therefore, the arginine stimulation test might be helpful in discriminating glucose-induced insulin-secretion defects from insulin-production defects in patients with disturbed glucose metabolism. Alternatively, the findings may indicate that, at least in earlier stages of the disease, the insulin-secretion defect is restricted to secretagogues requiring intact mitochondrial function.

Arginine not only stimulates the secretion of insulin from β-cells but also stimulates the release of glucagon from α-cells. In our study, the glucagon response to arginine did not differ between the patients with MIDD and the control subjects. These results are at variance with the findings in patients with type 1 and type 2 diabetes, who show an exaggerated glucagon response to arginine compared with nondiabetic control subjects (27). It has been clearly demonstrated that hypoinsulinemic diabetic patients have higher glucagon responses to arginine (28) and that improved blood glucose control decreases the exaggerated glucagon response to arginine in diabetic patients (29,30). One might expect, therefore, a normal glucagon response in our patients who showed good and tight blood glucose control with "normal" baseline insulin levels.

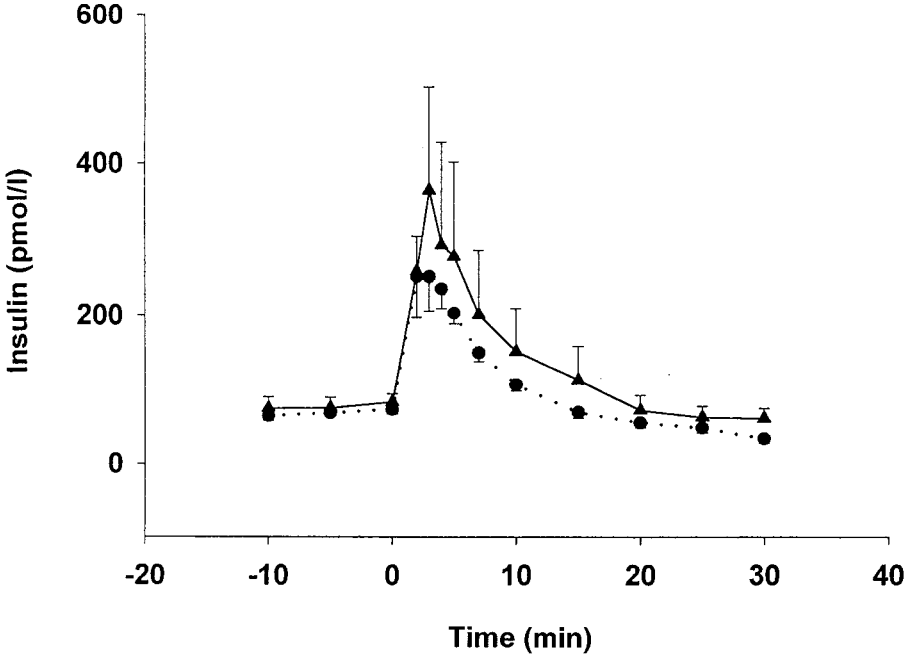

Figure 3— *Insulin response to arginine. ▲, patients; ●, controls.*

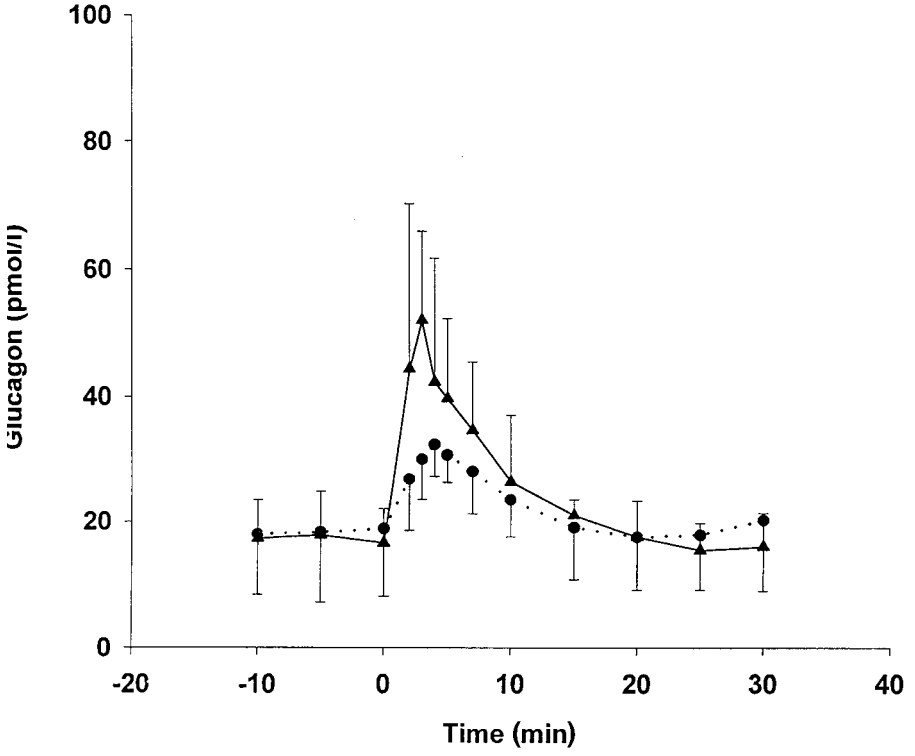

Figure 4— *Glucagon response to arginine. ▲, patients; ●, controls.*

These findings, however, are in contrast to the results from Kishimoto et al. (16), who studied five patients from different families with mitochondrial diabetes. These patients showed a blunted glucagon secretion on stimulation with arginine, suggesting that impaired α-cell function may be a characteristic feature of mitochondrial diabetes (16). Both our patients with MIDD and our study design differ from Kishimoto et al.'s subjects and study in several ways: *1*) our patients are from the same family and thus represent a more homogeneous patient population; 2) their duration of diabetes is much shorter (5 vs. 19 years); 3) our patients have excellent blood glucose control (HbA_{1c} 6.4 ± 0.4%) and no diabetes late complications, unlike Kishimoto et al.'s patients; and *4*) we injected a bolus of 5 g arginine over 30 s in contrast to an infusion of 30 g arginine over 30 min, as used by Kishimoto et al. Our results demonstrate that acute glucagon response to an arginine bolus (presumably via membrane depolarization) is normal in the early stages of well-controlled MIDD. Reduction of pancreatic islet mass with reduced oxidative potential after a long disease duration might also affect the α-cell function.

In summary, impaired insulin response to glucose in patients with mitochondrial $tRNA^{LEU(UUR)}$ gene mutation is an early and critical abnormality in the development of diabetes, whereas insulin and glucagon secretory responses to arginine are not affected at early stages of the disease.

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