Diabetes Mellitus Associated With the 3243 Mitochondrial tRNA$^{Leu(UUR)}$ Mutation: Insulin Secretion and Sensitivity

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To investigate the pathophysiology of diabetes mellitus associated with the 3243 mitochondrial tRNA$^{Leu(UUR)}$ mutation (DM-Mt3243), insulin secretion and sensitivity were studied using the 75-g oral glucose tolerance test (oGTT), 1-mg intravenous glucagon test, and euglycemic glucose clamp test. Twelve DM-Mt3243 patients were investigated (seven men and five women). Their ages ranged from 36 to 74 years, and the onset of diabetes occurred at 44.5 ± 9.5 years (mean ± SD). In the glucose tolerance test, nine patients (75.0%) showed lower C-peptide reactivity (CPR) than normal at 30 minutes, suggesting blunted insulin secretion. Three patients showed an impaired glucose tolerance (IGT) pattern, although they had absolute hyperglycemia at the onset of diabetes. In the glucagon test, 10 patients (76.3%) had CPR within the normal range at 6 minutes, indicating an adequate response. In the glucose clamp test, the M value was 8.70 ± 2.35 mg/kg/min and was within normal limits in all patients. The glucose metabolized (M value) was negatively correlated with 24-hour urinary C-peptide excretion ($r = .696$, $P < .05$). Thus, plasma CPR to glucose loading was blunted in many DM-Mt3243 patients, but CPR to glucagon was relatively well preserved. This difference in the intrinsic insulin response to the two stimuli may be characteristic of DM-Mt3243. Although M values were normal in all subjects, the correlation with 24-hour urinary C-peptide excretion suggests a relationship between insulin sensitivity and insulin secretion. These two mechanisms may cooperate to maintain homeostasis in this disease. Since three patients did not progress with aging, this mutation may not always cause gradual β-cell destruction.

MITOCHONDRIAL gene abnormalities cause various mitochondrial diseases and are often associated with diabetes. In 1992, van den Ouweland et al[1] reported pedigrees with maternal transmission of diabetes and identified the same 3243 mitochondrial DNA (mtDNA) mutation as in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS).[1] Because their patients had a different clinical picture from MELAS, diabetes mellitus associated with mitochondrial tRNA mutation at position 3243 (DM-Mt3243) is regarded as a new disease entity. However, the mechanism of association of this mutation with glucose intolerance has not been fully clarified. The roles of insulinopenia and peripheral insulin resistance are still controversial.[2-5]

In this study, 12 patients with DM-Mt3243 were investigated to clarify the pathophysiology of this form of mitochondrial diabetes by assessing several parameters related to glucose intolerance.

SUBJECTS AND METHODS

Subjects

Twelve diabetic patients with the 3243 mutation were studied. At diagnosis, all of the patients fulfilled World Health Organization (WHO) criteria for diabetes.[6] All were unrelated outpatients treated at Saiseikai Central Hospital, and were recruited by routine screening for the 3243 mtDNA mutation at our clinic. All subjects provided full informed consent to the study, which was approved by our institutional review committee.

Patients were excluded if they could not receive a 75-g oral glucose tolerance test (oGTT) and a glucagon challenge test. A euglycemic-hyperinsulinemic clamp test was also performed in nine subjects, but the other three (cases no. 8, 10, and 12 in Table 1) refused the clamp test.

Clinical profiles of the 12 subjects are summarized in Table 1. The subjects were seven men and five women aged 36 to 74 years (mean ± SD, 54.8 ± 12.4). None had typical neuromuscular manifestations of MELAS. Seven patients had mothers with diabetes and two had mothers with impaired glucose tolerance (IGT). None had a history of paternal diabetes. The age of onset of diabetes ranged from 31 to 58 years (44.3 ± 9.5). Body mass index (BMI) was 22.6 ± 5.5 kg/m^2. Eight patients were on insulin therapy, one was on oral hypoglycemic agent therapy (gliclazide), and the remaining three were on diet alone. Cases no. 1 and 3 had high insulin autoantibodies under insulin treatment. None had anti-GAD antibodies. Twenty-four-hour urinary C-peptide excretion ranged from 25 to 110 μg/d (Table 1).

Eleven subjects had hearing impairment on audiometry assessed by an otolaryngologist. Cases no. 4 and 6 presented with ketosis and hyperglycemia of abrupt onset. However, they were older than is typical for insulin-dependent diabetes mellitus (IDDM). They were later stabilized by insulin therapy without evidence of decreasing insulin secretion. The two patients showed negativity for islet cell antibodies, insulin autoantibodies, and anti-GAD antibodies. Accordingly, these two subjects could not be categorized as having typical IDDM.

Cases no. 2, 3, 4, and 7 were previously reported as having diabetic amyotrophy, insulin edema, posttreatment neuropathy, and atonic bladder, respectively.[7-10]

Mitochondrial DNA Assay

DNA was isolated from peripheral blood leukocytes or muscle. Mitochondrial DNA fragments encompassing position 3243 were amplified using polymerase chain reaction (PCR). The sense primer was 5′-AGGACAAGAGAAATAAGGCC-3′ (3130 to 3149) and the antisense primer 5′-CACGTTGGGGCCTTTGCGTA-3′ (3423 to 3404). [^{32}P]dATP was added to the PCR mixture to label the product. The

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Table 1. Clinical Profile of the Subjects

Case No.	Sex	Type of Diabetes	Family Member (DM or IGT)	Age (yr)	Onset of DM (yr)	Height (cm)	Weight (kg)	BMI	HbA_{1c} (%)	Therapy	Abnormal Audiogram	Retinopathy	Albumin Excretion Rate (mg/min)	24-Hour Urinary C-Peptide Excretion (μg/d)	Age at Start of Insulin (yr)	Duration of Insulin Treatment (yr)	IRI (pmol/L) Free	IRI (pmol/L) Total	Anti-GAD Antibody
1	F	NIDDM	Mother DM	63	52	153	72	30.8	8.0	Insulin	+ (high)	Back	3.8	68	59	4	126	360	—
2	M	NIDDM	Mother DM	74	50	162	40	15.2	7.5	SU	+ (total)	—	8.1	70	—	—	55	84	—
3	M	NIDDM	*	53	42	171	61	20.9	7.1	Insulin	+ (high)	Back	25	26	46	7	66	660	—
4	F	IDDM	*	36	31	149	35	15.8	8.0	Insulin	+ (total)	Back	5	25	31	5	78	78	—
5	F	NIDDM	Mother DM	50	38	163	55	20.7	8.2	Insulin	+ (high)	—	9.3	70	39	11	84	108	—
6	M	IDDM	Mother DM	39	34	158	65	26.0	9.0	Insulin	+ (low)	—	16.6	94	35	4	156	168	—
7	M	NIDDM	Mother DM	62	38	174	66	21.8	10	Insulin	+ (high)	Back	88.8	70	55	7	66	66	—
8	M	NIDDM	—	45	38	162	54	20.6	9.6	Insulin	+ (high)	—	3.8	39	39	6	150	150	—
9	F	NIDDM	—	70	58	150	52	23.1	9.2	Insulin	+ (high)	—	30	110	69	1	120	132	—
10	M	NIDDM	Mother DM	62	57	155	82	34.1	6.2	Diet	–	—	44	97	—	—	78	78	—
11	M	NIDDM	—	42	39	154	48	20.2	6.7	Diet	+ (high)	—	105	47	—	—	49	66	—
12	F	NIDDM	Mother DM	61	55	156	54	22.2	6.1	Diet	+ (high)	—	20.8	30	—	—	52	72	—
Mean ± SD				55 ± 12	44 ± 10	159 ± 8	57 ± 13	22.6 ± 5.5	8.0 ± 1.3				30.0 ± 33.7	62 ± 29			87 ± 38	151 ± 175	

Abbreviations: M, male; F, female; DM, diabetes mellitus; SU, sulfonylurea; high, hearing loss at high frequency; total, hearing loss at all frequencies; Back, background retinopathy.
*Mother showed IGT in the oGTT.

resultant 294-bp mitochondrial DNA fragments (3130 to 3423) were digested with a restriction endonuclease, *Apa*I, which recognizes the site of the A to G substitution (GAGCCC to GGGCCC). The fragments were then analyzed by electrophoresis on 0.8% agarose gel followed by autoradiography.[11,12]

Endocrinological Investigations

oGTT. All subjects began the oGTT at 8:30 AM after an overnight fast. They ate before 8:00 PM on the day before the test. Patients on insulin therapy received insulin injections until the morning of the day before the test. One patient on a oral hypoglycemic agent discontinued it from 1 day before the test. The patients fasted overnight and discontinued injections or oral hypoglycemic agents on the morning of the test. Intrinsic insulin secretion was observed by measuring plasma C-peptide reactivity (CPR).

Plasma glucose was assayed using the glucose oxidase method. Plasma CPR was assayed using a C-peptide radioimmunoassay kit developed by Shionogi (Tokyo, Japan). Serum immunoreactive insulin (IRI) was assayed using the double-antibody EIA kit developed by Boehringer Mannheim (Tokyo, Japan). The data are shown as free insulin.

Glucagon infusion test. For the glucagon infusion test, patients were studied after an overnight fast. Venous blood samples were taken 0 minutes before and 6 minutes after an intravenous bolus injection of 1 mg porcine glucagon (Novo Nordisk, Bagsvaerd, Denmark).[13]

Glucose clamp study. The euglycemic-hyperinsulinemic glucose clamp test was performed with an artificial pancreas apparatus (STG-22; Nikkiso, Tokyo, Japan) according to the method of Zuniga-Guajardo et al.[14] After an overnight fast, an indwelling catheter was inserted into an antecubital vein for glucose and insulin administration. A second catheter for blood sampling was inserted in a retrograde fashion into a superficial hand vein in the contralateral arm. The hand was kept in a warming chamber at 69°C to ensure arterization of venous blood. Insulin infusion (Novolin R; Novo-Nordisk, Copenhagen, Denmark) was started and maintained at a rate of 4.48 mU/kg body weight/min. Subsequently, various amounts of 20% dextrose were infused over 90 minutes to achieve a steady plasma glucose level of 5.6 mmol/L. In our preliminary study of diabetes, the mean plasma insulin concentration achieved during the steady-state portion of the clamp was 279 ± 72 μU/mL (N = 250 subjects).[15,16]

The glucose metabolized (M value) during the final 20 minutes of the glucose clamp was calculated based on the amount of glucose infused and expressed as milligrams per kilogram body weight per minute. In the presence of constant euglycemia, all infused glucose is taken up by the cells, thus indicating the amount of glucose metabolized by the entire body in response to infused insulin.

Statistics

Data are given as the mean ± SD. Spearman's correlation coefficient was calculated for the relationship between the M value and the clinical parameters. M values were compared by Student's *t* test. All *P* values are two-tailed.

RESULTS

75-g oGTT

The 75-g oGTT was performed in all 12 DM-Mt3243 patients. Nine patients (75.0%, cases no. 1 to 9) showed a 2-hour blood glucose level over 11.1 mmol/L (absolute hyperglycemia compatible with a diabetic pattern according to WHO criteria). However, the remaining three (cases no. 10 to 12) showed 2-hour levels lower than 11.1 mmol/L (mild hyperglycemia compatible with IGT pattern according to WHO criteria),

although they had absolute hyperglycemia at the onset of diabetes (Table 2).

Among the 12 patients, nine (75.0%) showed CPR at 30 minutes that was less than normal (normal range at 30 minutes, 1.06 to 2.75 nmol/L; mean ± SD, 1.66 ± 0.46 nmol/L[17]), and seven (58.3%) showed CPR at 60 minutes that was less than normal (normal range at 60 minutes, 1.20 to 2.88 nmol/L; mean ± SD, 1.89 ± 0.50 nmol/L[17]). Thus, about 60% to 75% of DM-Mt3243 showed blunted early intrinsic insulin secretion.

Glucagon Infusion Test

A glucagon infusion test was performed for all 12 DM-Mt3243 patients. Plasma CPR after 6 minutes of infusion was 1.26 ± 0.43 nmol/L. CPR at 6 minutes was within normal limits in 10 (83.3%) patients (normal range at 6 minutes, 0.89 to 2.75 nmol/L; mean ± SD, 1.50 ± 0.50 nmol/L[17]). Thus, 10 patients (76.3%) showed an adequate response to glucagon challenge (Fig 1).

Euglycemic Clamp Test

M values in nine patients with DM-Mt3243 who underwent the clamp test ranged from 5.47 to 12.61 mg/kg body weight/min. The mean M value was 8.70 ± 2.35 mg/kg body weight/min (Table 2).

By our preliminary study, the M value obtained in 13 healthy controls matched for age and BMI (age, 55.3 ± 14.6 years; BMI, 23.2 ± 3.6 kg/m^2) was 9.53 ± 3.45 mg/kg body weight/min (range, 5.18 to 13.12; T. Iizuka and T. Nishikawa, unpublished data, March 1996). Hence, all nine patients with DM-Mt3243 showed M values within the normal range, and there was not a statistical difference in M values between healthy controls and DM-Mt3243 patients.

Table 2. Results of the 75-g oGTT and M Value in the Glucose Clamp

Case No.		Time After Glucose Loading (min) 0	30	60	90	120	180	M Value in Glucose Clamp Test (mg/kg/min)
Diabetes pattern								
1	Plasma glucose	12.3	17.6	22.6	25.4	25.1	19.3	7.62
	Serum IRI	185	234	278	310	292	273	
	Plasma CPR	0.50	0.79	1.13	1.16	1.22	1.20	
2	Plasma glucose	8.2	15.7	17.9	18.8	21.7	19.2	8.75
	Serum IRI	28	90	100	91	88	61	
	Plasma CPR	0.50	1.19	1.32	1.52	1.75	1.49	
3	Plasma glucose	9.6	16.9	21.0	20.3	21.2	19.0	9.20
	Serum IRI	47	76	68	58	62	60	
	Plasma CPR	0.36	0.70	0.86	0.63	0.66	0.66	
4	Plasma glucose	5.7	12.6	18.4	13.9	11.8	10.5	11.74
	Serum IRI	83	161	130	121	94	72	
	Plasma CPR	0.20	0.83	0.83	0.76	0.66	0.46	
5	Plasma glucose	5.2	10.5	13.5	16.2	15.9	14.7	5.47
	Serum IRI	82	174	268	273	150	124	
	Plasma CPR	0.43	0.70	1.06	1.52	1.06	0.89	
6	Plasma glucose	6.7	13.2	19.1	19.9	18.2	12.7	8.09
	Serum IRI	68	106	226	280	233	161	
	Plasma CPR	0.23	0.56	1.46	2.15	2.05	1.52	
7	Plasma glucose	10.5	13.6	19.6	22.0	23.6	19.3	8.85
	Serum IRI	23	44	53	44	61	49	
	Plasma CPR	0.50	0.63	0.76	0.79	0.86	0.83	
8	Plasma glucose	7.5	14.7	18.2	20.8	21.1	20.7	ND
	Serum IRI	44	58	58	72	69	53	
	Plasma CPR	0.20	0.36	0.43	0.60	0.70	0.63	
9	Plasma glucose	6.2	12.0	11.7	12.9	14.8	7.9	5.99
	Serum IRI	56	177	294	312	297	152	
	Plasma CPR	0.56	1.66	2.45	2.85	3.31	2.35	
IGT pattern								
10	Plasma glucose	5.7	9.3	9.3	8.8	9.4	8.7	ND
	Serum IRI	40	268	268	329	319	277	
	Plasma CPR	0.83	2.22	2.42	2.91	3.31	3.28	
11	Plasma glucose	6.0	8.1	10.6	9.8	9.3	3.7	12.61
	Serum IRI	12	118	278	150	110	61	
	Plasma CPR	0.36	0.70	1.06	1.52	1.06	0.89	
12	Plasma glucose	5.0	10.4	12.3	12.9	10.8	6.1	ND
	Serum IRI	40.8	135	221	346	254	146	
	Plasma CPR	0.43	0.89	1.39	2.25	2.48	2.02	

NOTE. Normal plasma C-peptide excursion is 1.4 ± 0.4 (mean ± SD; range, 0.7-2.2) before the test, 5.0 ± 1.4 (3.2-8.3) at 30 minutes, 5.7 ± 1.5 (3.6-8.7) at 60 minutes, 5.3 ± 1.6 (3.3-9.4) at 90 minutes, 5.6 ± 1.8 (3.3-9.0) at 120 minutes, and 3.9 ± 1.9 (1.5-8.4) at 180 minutes. Units: glucose, mmol/L; serum IRI, pmol/L; plasma CPR, nmol/L; M value, mg/kg body weight/min. ND indicates that the patient did not undergo the glucose clamp study.

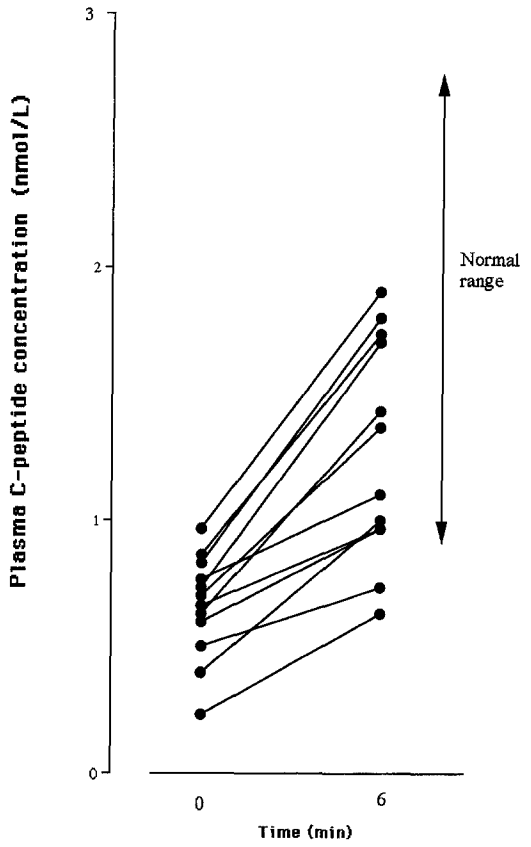

Fig 1. Plasma C-peptide concentrations at 0 and 6 minutes after the 1-mg intravenous glucagon challenge.

On the other hand, the M value for 530 NIDDM patients matched for age and BMI (age, 56.6 ± 9.1 years; BMI, 22.9 ± 4.3 kg/m^2) was 6.88 ± 2.54 mg/kg body weight/min (T. Iizuka and T. Nishikawa, unpublished data), which was less than that for DM-Mt3243 subjects ($P < .05$).

In our DM-Mt3243 subjects, the M value was not correlated with the parameters in Table 1 such as fasting plasma glucose, age, onset of diabetes, BMI, HbA_{1c}, and albumin excretion rate, but it was negatively correlated with 24-hour urinary C-peptide excretion ($r = .696$, $P < .05$) (Fig 2).

DISCUSSION

In the present study, many DM-Mt3243 patients showed blunted early insulin secretion on oGTT. This finding confirms the assumption that impairment of the intrinsic insulin response to glucose stimulation is central to the pathogenesis of hyperglycemia in DM-Mt3243, supporting previous observations.[2,3,12] However, our patients on insulin therapy received insulin injections until the morning of the day before the test, suggesting that the residual effect of extrinsic insulin might not have been excluded completely. Hence, we cannot deny the possibility that the underlying effect of a relatively high insulin concentration contributed to the reduced C-peptide response to glucose.

In contrast to glucose, CPR to glucagon was preserved in a high percentage of patients. Because the glucagon effect within the early phase after bolus injection reflects the glucagon effect rather than the glucose effect, this difference between the two challenge tests may represent a different signaling point for pancreatic insulin secretion. Because glucagon directly increases cAMP in β cells, a high intracellular ATP/ADP ratio can be achieved. This suppresses ATP-sensitive K channels, elevates cytosolic Ca^{2+}, and promotes intrinsic insulin secretion, possibly irrespective of mitochondrial dysfunction.[18]

Our result was in contrast to the data from Kishimoto et al,[2] who reported that five of their DM-Mt3243 patients did not show a marked CPR increase after glucagon. Therefore, the response to glucagon stimuli may not always be intact in DM-Mt3243. Nevertheless, their patients had a younger age of onset of diabetes (28.5 ± 13.7 years) than our patients. Since the genetic contribution to pathogenesis in mitochondrial disease is supposed to be greater in those presenting at a early age versus an older age, the deleterious effect of the 3243 mutation might originally have been stronger in their group than in our subjects. Consequently, in their group, the destruction of pancreatic β cells might have progressed further and eventually blunted the CPR to glucagon.

Because mutant mtDNAs may be present in muscle and fat tissue, insulin resistance can theoretically be related to the defective mitochondrial function.[4,19] However, in the present series, all subjects examined in the clamp study showed M values within the normal range. Compared with the data from NIDDM patients, the M value for our DM-Mt3243 subjects was high, suggesting a relatively increased insulin sensitivity in the population of diabetics.

Using the normal criteria range of 24-hour urinary C-peptide excretion (60 to 120 μg/d), seven patients of our 12 DM-Mt3243 subjects had normal values and five had low values. In addition, interestingly, the M value was negatively correlated with 24-hour urinary C-peptide excretion. This suggests relatively increased insulin sensitivity in patients with low insulin secretion. We speculate that pancreatic insulin secretion and peripheral insulin sensitivity may cooperate to maintain glucose

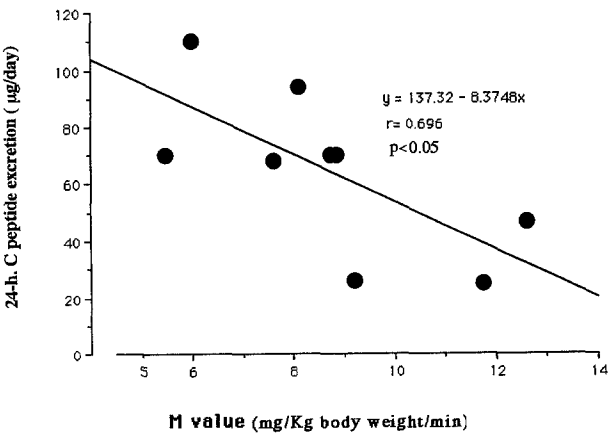

Fig 2. Correlation between the M value determined with the hyperinsulinemic-euglycemic glucose clamp and 24-hour urinary C-peptide excretion. A significant negative correlation was noted ($r = .696$, $P < .05$) between the variables, indicating a relationship between insulin sensitivity and insulin secretion.

homeostasis. It is possible that the disruption of this cooperation can induce the worsening of hyperglycemia and facilitate the development of diabetes.

Many groups have stated that DM-Mt3243 is progressive. Katagiri et al[20] observed that their DM-Mt3243 patients had a gradual increase of the insulin requirement with aging. In this study, eight (66.6%) of 12 patients progressed to an insulin-requiring state. This proportion is high compared with ordinary Japanese NIDDM patients. Two theories are believed to explain such progression: (1) mutant genomes predominate over the wild-type with aging, and (2) cumulative damage by free radicals and reactive oxygen may cause time-dependent worsening of mitochondrial function.[21]

However, three of our patients (cases no. 10 to 12) showed improvement on the oGTT to an IGT pattern. Hence, some DM-Mt3243 patients can improve with time. This is clinically important because it may give hope for a better prognosis to affected patients. Such patients might have originally had a minor influence of genetic factors on the pancreas, but tentatively developed hyperglycemia under given conditions such as alcohol abuse, tobacco use, or vitamin deficiency.[22] Because the original damage was mild, such patients might then overcome the disruption of glucose homeostasis in response to the effect of better glycemic control on β-cell function. For the worsening factor, the role of alcohol abuse seemed important in our case no. 11. Interestingly, he was an alcohol drinker before the onset of diabetes; however, after stopping alcohol, glycemic control improved rapidly without adherence to a diet.[23]

In conclusion, plasma CPR secretion in response to glucose was blunted in many DM-Mt3243 patients, but in response to glucagon it was relatively preserved. This different intrinsic insulin response to the two stimuli may reflect the different signaling in the pancreas and be characteristic of DM-Mt3243. M values were normal in all subjects examined, but the correlation with 24-hour urinary C-peptide excretion suggests possible cooperation between insulin sensitivity and insulin secretion. In addition, not all patients progressed with aging, which suggests that this mutation does not always cause gradual β-cell destruction. Thus, these three findings provide hints to understanding the underlying pathophysiology of DM-Mt3243.

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