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A Case of Myotonic Dystrophy (MD) Associated with Glucose-Induced Hyperinsulinemia Followed by Reactive Hypoglycemia and Increased Number of Cytosine-Thymine-Guanine (CTG) Trinucleotide Repeats in MD Gene

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Abstract. A 39-year-old man with myotonic dystrophy consulted our hospital for nausea, vomiting and dizziness that occurred after 75 g oral glucose tolerance test (OGTT). Reexamination of OGTT revealed remarkable hyperinsulinemia (622 μU/ml) followed by reactive hypoglycemia (50 mg/dl) and such hypoglycemic symptoms as nausea, vomiting, dizziness and palpitation. DNA analysis of the circulating lymphocytes revealed increased (1,500 times) number of cytosine-thymine-guanine (CTG) trinucleotide repeats in myotonic dystrophy protein kinase (DM kinase) gene. Gel chromatographic analysis of the plasma in combination with sensitive enzyme immunoassay of insulin revealed that the ratio of proinsulin to total immunoreactive insulin was elevated at fasting (12.9%), and was decreased to 8.9% at 60 min after glucose administration. These findings may indicate that biologically active authentic insulin was predominantly secreted after glucose administration in the present case. This is the first case report of myotonic dystrophy with hyperinsulinemia associated with reactive hypoglycemia induced by oral glucose administration.

Key words: Myotonic dystrophy, Reactive hypoglycemia, Oral glucose administration, Proinsulin, Hyperinsulinemia

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MYOTONIC dystrophy is a muscular disease of autosomal dominant inheritance, which is characterized by muscle weakness and atrophy, cataract, myocardial disorders, respiratory disorders and mental retardation, and also associated with such endocrine disorders as insulin resistance, impaired pituitary hormone secretion and primary male hypogonadism [1]. Myotonic dystrophy is accompanied by hyperinsulinemia after oral glucose administration [2], whereas basal plasma insulin levels remain within the normal range [3, 4]. In spite of remarkable hypersecretion of insulin, there have been no reports on reactive hypoglycemic episode in patients with myotonic dystrophy.

Recently, myotonic dystrophy gene was identified [5], indicating that myotonic dystrophy was caused by an increased number of cytosine-thymine-guanine (CTG) trinucleotide repeats in the 3′ untranslated region of a protein kinase gene located on the q13.3 band of chromosome 19 [6, 7]. Furthermore, a positive correlation was shown between the increased number of CTG repeats and the severity of neuromuscular symptoms or earlier onset [8–11]. However, there remains to be elucidated the possible

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relationship between insulin secretion and CTG repeats.

We report the first case of myotonic dystrophy associated with reactive hypoglycemia after oral glucose administration and remarkably increased CTG repeats in the protein kinase gene. We also demonstrated that the increased ratio of proinsulin to total insulin immunoreactivity was rather decreased after glucose administration in the patient's plasma by gel chromatographic analysis combined with sensitive enzyme immunoassay of insulin.

Case Report

A 39-year-old man was referred to our hospital for further examination of nausea, vomiting and dizziness which occurred after 75 g oral glucose administration. He was diagnosed as having a waddling gait and right walleye at the age of 7 yrs and 17 yrs, respectively. Aropetia appeared on the frontal and occipital lesion at 25 yrs, and muscle weakness of the extremities appeared at 34 yrs. He then consulted our hospital and was diagnosed as myotonic dystrophy. At the age of 39 yrs, nausea, vomiting and

Table 1. Laboratory Data on Admission

CBC			Biochemistry			Renal function		
WBC	8,300	/μl	TP	6.6	g/dl	24 h Ccr	122	l/day
Seg	56	%	Alb	4.3	g/dl	u-Alb	5	mg/day
Baso	1	%	T. Bil	0.6	mg/dl	u-β2MG	32	μg/day
Lymph	38	%	GOT	22	IU/l			
Mono	5	%	GPT	27	IU/l	Chromosome		
RBC	463×10^4	/μl	CPK	174	IU/l	46XY, normal karyotype		
Hb	14.7	g/dl	ALP	62	IU/l			
HT	43.8	%	LAP	36	IU/l			
Plt	27.5×10^4	/μl	LDH	371	IU/l	Endocrine		
			r-GTP	16	IU/l	GH	0.3	ng/ml
Urinalysis			ChE	268	IU/l	IGF-I	190	ng/ml
pH	5.5		T. Cho	139	mg/dl	LH	8.6	mIU/ml
Pro	(−)		TG	126	mg/dl	FSH	23.7	mIU/ml
Glu	(−)		NEFA	0.61	mEq/l	Testosterone	483.0	ng/dl
Blood	(−)		HDL-cho	37	mg/dl	T3	129	ng/dl
Bil	(−)		BUN	11	mg/dl	T4	9.7	μg/dl
Uro	(±)		Crea	0.6	mg/dl	Free T4	1.2	ng/dl
Sediment	(−)		UA	4.4	mg/dl	TSH	2.99	μU/ml
			Na	144	mEq/l	PRL	4.2	ng/ml
			K	3.8	mEq/l	ACTH	34	pg/ml
			Cl	106	mEq/l	Cortisol	9	μg/dl
			Ca	9.0	mg/dl	u-17OHCS	5.8	mg/day
			Fe	67	μg/dl	u-17KS	2.4	mg/day
			FBS	78	mg/dl	ADH	1.0	pg/ml
			HbA1c	5.0	%			

Endogenous insulin secretion					
CPR	2.0	ng/ml			
Urine CPR	48	μg/day			
Plasma glucagon	65	pg/ml			
Glucagon Loading Test					
Time (min)	0	4	6	8	10
Plasma Glucose (mg/dl)	72	79	78	98	104
CPR (ng/ml)	2.0	6.6	7.0	9.9	11.2
Euglycemic Glucose Clamp					
Glucose Infusion Rate (mg/kg/min)	6.01				

dizziness were recognized 3 hours after oral administration of 75 g glucose. There was no family history of myotonic dystrophy or diabetes mellitus.

His height was 169 cm and weight was 53 kg, body mass index was 18.6 kg/m^2, body temperature was 36.5°C, blood pressure was 108/74 mmHg, and pulse rate was 88/min and regular. Aropetia of forehead and right walleye were recognized. Muscles in peripheral lesion were atrophic. There was no goiter or lymphadenopathy. Heart and lung were normal. Liver and kidney were not palpable. Grasping power was lowered to 13 kg for the right and 12 kg for the left. His intelligent quotient was 59. Electromyography showed a dive bomber sound-like appearance.

Laboratory findings are shown in Table 1. Urinalysis and blood biochemistry data were normal. Endocrinological data and atrophic testis showed latent primary hypogonadism. Endogenous insulin secretion evaluated by plasma and urinary C-peptide immunoreactivity (CPR) levels were within the normal range, but glucagon administration (1 mg, iv) resulted in a hyperresponse of plasma CPR. Euglycemic glucose clamp study showed decreased glucose infusion rate (GIR) (6.01 mg/kg/min vs. normal range: 6.2–9.8 mg/kg/min), suggesting slightly increased insulin resistance [12].

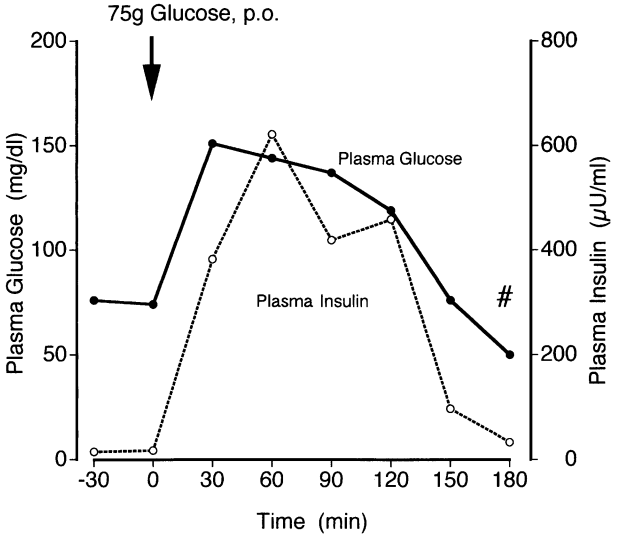

Fig. 1. Effect of 75 g oral glucose administration on plasma glucose levels (closed circle), plasma immunoreactive insulin levels (open circle) in a patient with myotonic dystrophy. # shows episodic appearance of nausea and dizziness.

As shown in Fig. 1, plasma glucose levels for 120 min after oral administration of 75 g glucose were considered as the normal pattern, assessed by both the diagnostic criteria of American Diabetes Association 1997 and Japan Diabetes Society 1999. On the other hand, plasma insulin level was remarkably increased to 622 μU/ml at 60 min after the glucose administration, although basal plasma insulin level was 17 μU/ml. The patient complained of nausea and dizziness at 180 min after the start of glucose administration, when plasma glucose level was 50 mg/dl. Informed consent to gene analysis and oral administration of 75 g glucose was obtained from the patient.

Myotonic dystrophy protein kinase (DM kinase) DNA analysis

DNA was extracted from circulating lymphocytes and analyzed for the number of CTG repeats in DM kinase as previously described [6, 7]. DNA was digested with either Eco RI or Bgl I, and electro-

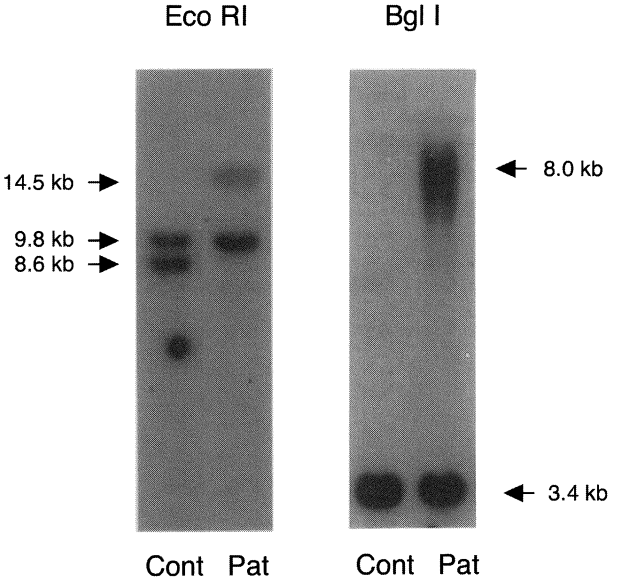

Fig. 2. Myotonic dystrophy protein kinase DNA analysis. DNA was digested with Eco RI (left panel) or Bgl I (right panel) and electrophoresed on agarose gels. DNA fragments were transferred onto membranes, which were then hybridized with the digoxigenin-labeled cDNA25 probe. The abnormal bands were visualized at 14.5 kb and 8.0 kb by chemiluminescence method. Cont and Pat indicated normal control and the present patient, respectively.

phoresed on agarose gels. DNA fragments were transferred onto membranes, which were then hybridized with the digoxigenin-labeled cDNA25 probe. The bands were visualized by chemiluminescence method.

As shown in Fig. 2, the abnormal bands were recognized at 14.5 kb and 8.0 kb after treatment with Eco RI and Bgl I, respectively. The band of 14.5 kb appeared as a smear of varying intensity. This suggested that it showed somatic mosaicism due to mitotic instability in lymphocytes [6]. The number of CTG repeats calculated in the patient with myotonic dystrophy was remarkably increased to as much as 1,500 times (normal: <30 times).

Gel chromatographic analysis and measurement of immunoreactive insulin by enzyme immunoassay

Plasma samples obtained from a patient with myotonic dystrophy were analyzed by gel chromatography. Five-hundred μl of each sample was fractionated on a Sephadex G-50 column (1.0×95 cm) (Pharmacia Co., Sweden), which was calibrated with blue dextran (2,000 K daltons), cytochrome c (12.4 K daltons) (Sigma, USA) and recombinant human insulin (Humalin R, Shionogi Co., Japan). The column was eluted at a rate of 10 ml/hr at 4°C using 0.01 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl and 0.1% BSA, and fractionated in a volume of 1.0 ml. One hundred μl of each fraction was used for measurement of immunoreactive insulin in duplicate.

Anti-insulin serum was obtained in the guinea pig by multiple subcutaneous injections of porcine insulin (Sigma Co., St. Louis, MO, USA). Anti-porcine insulin Fab' was prepared by the method previously described [13, 14, 15], and then conjugated with horseradish peroxidase by the maleimide method. Anti-porcine insulin IgG coated polystyrene balls (3.2 mm in diameter, Immunochemical Inc., Okayama, Japan) were used as a solid phase.

For the measurement of immunoreactive insulin levels, polystyrene balls were incubated with 100 μl of standard or samples and 50 μl of assay buffer in duplicate at 37°C for 1 hr. After removal of the supernatant, the polystyrene balls were washed and then incubated with Fab'-peroxidase conjugate at 37°C for 1 hr. After washing, the balls were incu-

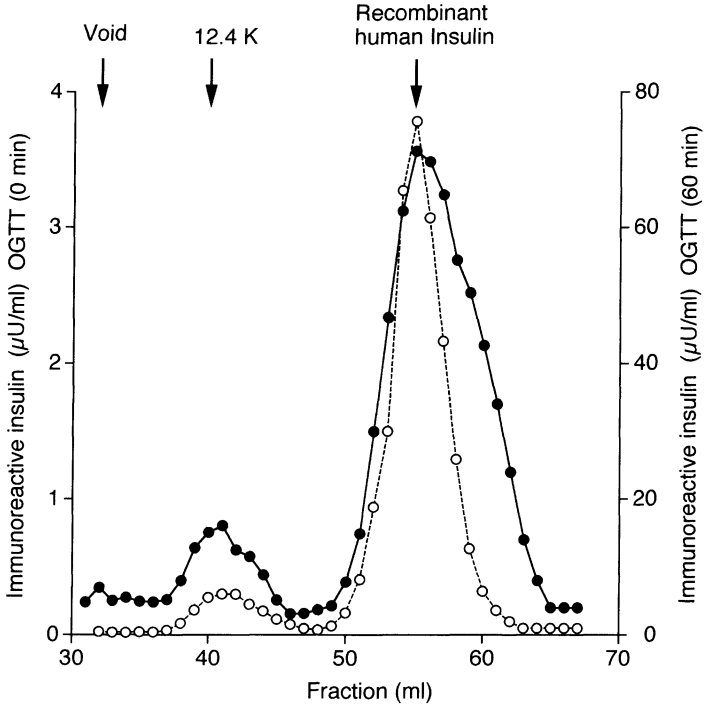

Fig. 3. Elution profiles of patient's plasma before (closed circle) and 60 min (open circle) after 75 g oral glucose administration. Five-hundred μl of plasma was applied on a Sephadex G-50 column (1.0×95 cm).

bated with 100 μl of 0.6% 3-(p-hydroxyphenyl) propionic acid as substrate and 50 μl of 0.015% H_2O_2 at 30°C for 60 min. The reaction was stopped by adding 0.1 M glycine-NaOH buffer, pH 10.3. Fluorescence intensity was measured by spectrofluorophotometer (Shimazu RF-5000, Shimazu Co., Kyoto, Japan).

The minimum detectable quantity was 0.15 μU /ml, and intra- and inter-assay coefficient of variance was 6.1% and 7.0%, respectively. Cross-reactivity of the enzyme immunoassay produced against porcine insulin was 100% for human insulin and proinsulin.

Fig. 3 shows the elution profile of plasma samples obtained before and 60 min after oral glucose administration. Two peaks of immunoreactive insulin were obtained in each plasma. The first small peak corresponded to proinsulin and the second large peak was insulin. The molar ratio of proinsulin to total immunoreative insulin was calculated as 12.9% before glucose administration, but it was rather decreased to 8.9% at 60 min after 75 g oral glucose administration.

Discussion

We investigated a patient with myotonic dystrophy associated with remarkable hyperinsulinemia and hypoglycemic attack after 75 g oral glucose administration. Reactive hypoglycemic attack is known to be caused by hypersecretion of insulin after oral glucose administration in patients with total gastrectomy [16], hyperthyroidism [17] and diabetes mellitus [18]. On the other hand, there has been no report on hypoglycemic attack after glucose administration in patients with myotonic dystrophy, suggesting an involvement of insulin resistance in the peripheral tissue.

Kakehi *et al.* [19] reported that basal plasma insulin levels were within the normal range in almost all cases with myotonic dystrophy in Japan, and that accumulated plasma insulin levels at 30, 60 and 120 min after glucose administration were evenly divided into three subgroups: 1) less than 150 μU/ml, 2) 151–300 μU/ml and 3) more than 301 μU/ml. Furthermore, there was no relationship among muscle atrophy, insulin secretion and impaired glucose tolerance. In the present case, basal plasma insulin level was slightly increased, suggesting insulin resistance. The accumulated plasma insulin level was 1463 μU/ml, one of the highest levels in past reports.

Krentz *et al.* [20] reported that there was no difference between fasting plasma insulin levels and C-peptide immunoreactivity, but proinsulin and 32–33 split proinsulin were increased. These findings suggest that processing of proinsulin to insulin is impaired in pancreatic beta cells in patients with myotonic dystrophy [20]. We analyzed the molar ratio of proinsulin to total immunoreactive insulin in the plasma using gel chromatography in the present case. Two major peaks of immunoreactive insulin were recognized: the smaller peak of proinsulin and the larger peak of authentic insulin. The ratio of proinsulin to total immunoreactive insulin was elevated (12.9%) at the fasting stage, which was compatible with previous reports [20]. On the other hand, the ratio of proinsulin to total immunoreactive insulin decreased to 8.9% after oral administration of glucose, suggesting that authentic immunoreactive insulin secretion was predominantly stimulated by glucose. Therefore, the reactive hypoglycemia might be induced by increased authentic insulin.

The detailed mechanism for hyperinsulinemia after oral glucose administration remains to be fully elucidated in myotonic dystrophy. This is mainly explained by insulin hypersecretion due to increased peripheral insulin resistance [21–25]. There have been few reports on the biological activity of endogenous insulin in myotonic dystrophy. Moxley *et al.* [26] reported that insulin action was not rapidly enhanced after oral glucose challenge in myotonic dystrophy. Insulin bioactivity was evaluated by using rat adipose tissue system [2].

On the other hand, another bioassay system of insulin revealed that 85–90% of immunoreactive insulin was recovered in plasma of the patient with myotonic dystrophy [27]. However, the presence of hypoglycemic attack as an indicator of insulin bioactivity *in vivo* has not been reported in patients with myotonic dystrophy. In the present case, insulin resistance in peripheral tissue was confirmed by glucose clamp, but hypoglycemic attack was clearly induced. These findings suggest that endogenous insulin secretion stimulated by glucose might overcome the degree of insulin resistance, or secreted insulin was biologically active enough to induce hypoglycemic attack in the present case.

There have been some reports on positive correlation between the increased number of CTG repeats and severity of neuromuscular symptoms or earlier onset [8–11]. However, there remains to be elucidated the relationship between insulin secretion and CTG repeats. Annane *et al.* [28] reported that the length of the expansion of CTG repeats correlated negatively with the brain glucose metabolism and positively with the area under the curve for insulin change after oral glucose administration. Further study is required to make clear the relationship between insulin secretion and genetic severity of CTG repeats.

Myotonic dystrophy associated with insulinoma has been reported in a patient, who showed hypoglycemic attack after meals [29]. However, in the present case there was no findings suggesting insulinoma.

In summary, we report the first case with myotonic dystrophy associated hyperinsulinemia and hypoglycemic attack induced by oral glucose administration, and remarkably increased number of CTG repeats in myotonic dystrophy gene.

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