Contribution of Abnormal Insulin Secretion and Insulin Resistance to the Pathogenesis of Type 2 Diabetes in Myotonic Dystrophy

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OBJECTIVE — Myotonic dystrophy (MyD), the most common adult form of muscular dystrophy, is often complicated by diabetes. MyD is dominantly inherited and is due to heterozygosity for a trinucleotide repeat expansion mutation in a protein kinase gene able to induce derangement of RNA metabolism responsible of an aberrant insulin receptor expression.

RESEARCH DESIGN AND METHODS — To assess insulin sensitivity and secretion before the onset of diabetes, we studied 10 MyD patients, 10 offspring of type 2 diabetes (OFF), and 10 healthy subjects with no family history of diabetes (control subjects) with dual X-ray energy absorption, euglycemic-hyperinsulinemic clamp (40 mU/[m^2 · min]) combined with infusion of [6,6-D_2]-glucose and oral glucose tolerance test (OGTT).

RESULTS — MyD had reduced lean body mass, but peripheral insulin sensitivity was not different to that of control subjects in contrast to OFF, which showed insulin resistance. Insulin secretion, obtained by deconvolution of OGTT data, was also shown to be comparable with that of OFF and control subjects (index of β-cell function = Φ; $P = 0.91$) even if increased parameters of insulin secretion were found during the first 30 min (Φ_{30}; $P = 0.05$) of the oral glucose challenge. Fasting plasma proinsulin concentrations ($P = 0.01$) and the ratio to insulin ($P = 0.01$) were increased in MyD patients. The proinsulin levels also failed to be suppressed during the clamp and showed exaggerated response after the OGTT. Increased proinsulin levels were shown to be peculiar of MyD patients when compared with OFF.

CONCLUSIONS — In nondiabetic, young MyD patients, insulin sensitivity was preserved, and an increased early secretory response to oral glucose was detected. Abnormal plasma proinsulin levels in the fasting state, during the clamp, and during the OGTT were shown to be secretory dysfunctions peculiar of MyD patients and may be more important than insulin resistance in determining the high risk to develop diabetes in these patients.

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Abbreviations: AUC, area under the curve; DEXA, dual energy X-ray absorption; LBM, lean body mass; MyD, myotonic dystrophy; OFF, study group of offspring of diabetic parents; OGTT, oral glucose tolerance test; PIM, proinsulin immunoreactivity; QUICKI, quantitative insulin sensitivity check index; $SIp_{(clamp)}$, clamp-derived index of insulin sensitivity.

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

Myotonic dystrophy (MyD) is a multisystemic, autosomal dominant disorder associated with progressive muscle wasting and weakness (1). The onset of symptoms occurs in the second and third decades of life, and it is the most common adult form of muscular dystrophy with an estimated prevalence of 1 in 8,000 (1). It is caused by heterozygosity for a trinucleotide repeat expansion mutation in the 3′-untranslated region of a protein kinase gene (DM kinase) located on the q13.3 band of chromosome 19 (2). Insulin resistance and hyperinsulinemia are considered severe metabolic abnormalities (3–7) also able to induce type 2 diabetes. In MyD, the CTG repeat is transcribed (mRNA) but not translated (protein), and a generalized disruption of RNA metabolism mediated by accumulation of abnormal RNA might cause the clinical syndrome (8). In particular it was observed that skeletal muscle of patients with MyD had reduced insulin receptor RNA and protein expression, and recently it was suggested that an alternative splicing of the insulin receptor premRNA is aberrantly regulated in MyD skeletal muscle (9). These abnormalities may represent a possible molecular mechanism responsible for insulin resistance (10). The aim of this study was to characterize the early defects of glucose/insulin homeostasis associated with MyD. We assessed body composition, insulin action, and insulin secretion in 10 young, nondiabetic patients with MyD using the insulin clamp and the oral glucose tolerance test (OGTT). We also compared them with healthy subjects carefully matched for anthropometric parameters with no family history of diabetes (control subjects) or with a first-degree relative with type 2 diabetes (offspring of diabetic parents [OFF]) to establish whether the possible alterations of glucose/insulin metabolism were peculiar of MyD patients or were common to the classical form of type 2 diabetes. An additional group of eight

Table 1—*Anthropometric parameters and body composition of study groups*

	MyD	OFF	Control subjects	Diabetic subjects
Patients (*n*, females/males)	8 F/2 M	8 F/2 M	8 F/2 M	5 F/3 M
Age (year)	38 ± 4	32 ± 2	33 ± 4	53 ± 3†
Body weight (kg)	60 ± 4	64 ± 5	67 ± 3	81 ± 4
Height (cm)	165 ± 3	171 ± 5	173 ± 2	170 ± 7
BMI (kg/m^2)	22.3 ± 1.4	21.9 ± 1.8	22.4 ± 1.2	27.1 ± 1.1†
Waist-to-hip ratio	0.77 ± 0.01	0.72 ± 0.04	0.73 ± 0.03	0.97 ± 0.04†
Ideal body weight (%)	104 ± 4	103 ± 5	104 ± 3	134 ± 12†
Body fat mass (kg)	22.1 ± 2.8	16.0 ± 3.1	17.4 ± 2.4	ND
Body fat (%)	34.1 ± 3.3*	26.0 ± 2.9	25.9 ± 2.6	ND
Arms fat content (%)	31.4 ± 3.4*	22.5 ± 4.1	21.9 ± 3.4	ND
Trunk fat content (%)	33.5 ± 3.2*	19.9 ± 4.1	21.3 ± 3.4	ND
Legs fat content (%)	38.0 ± 2.2	32.8 ± 3.2	32.1 ± 2.8	ND
LBM (kg)	35.6 ± 2.7†	45.7 ± 3.3	46.9 ± 3.0	ND
Physical activity index‡	10.2 ± 0.9	9.0 ± 0.5	8.9 ± 1.4	8.5 ± 0.9

Data are means ± SEM. ND, not determined. *$P < 0.05$ vs. OFF and control subjects. †$P < 0.02$ vs. OFF and control subjects. ‡The range of possible scores for the physical activity index is 3–15; the lowest value corresponds to the level of physical activity of a clerical worker who plays a light sport (energy expended is <0.76 MJ/h; e.g., bowling) and who participates in sedentary activities during leisure time. The highest value corresponds to the level of physical activity of a person who is very physically active at work (e.g., a construction worker), who plays heavy sports (energy expended is at least 1.76 MJ/h; e.g., boxing, basketball, football, or rugby), and who is very physically active during leisure time (e.g., walking >1 h/day or biking >45 min/day).

patients with type 2 diabetes at the onset of the disease were studied with the OGTT.

RESEARCH DESIGN AND METHODS

Subjects

Ten patients with classical adult-onset MyD were recruited in the Division of Neurology of the Istituto Scientifico H San Raffaele, and matched healthy volunteers served as control subjects. All control subjects were in good health as assessed by medical history, physical examination, hematological, and urinanalysis; subjects did ($n = 10$) or did not ($n = 10$) have family history of diabetes, obesity, and hypertension traced through their grandparents and had a sedentary lifestyle. MyD patients were selected and carefully matched with healthy subjects for anthropometric parameters to avoid a confounding effect of obesity (11). The clinical and laboratory characteristics of the three groups of subjects are summarized in Table 1. Informed consent was obtained from all subjects after explanation of purposes, nature, and potential risks of the study. The protocol was approved by the Ethical Committee of the Istituto Scientifico H San Raffaele.

Experimental protocol

Subjects were instructed to consume an isocaloric diet (~250 g of carbohydrate/day) and to abstain from exercise activity for 3 weeks before the studies. Women were studied between days 3 and 10 of the menstrual cycle. They were studied to assess whole body insulin sensitivity and endogenous glucose production after a 10-h overnight fast period and during the euglycemic-hyperinsulinemic clamp. The day after, they also underwent an OGTT performed following the American Diabetes Associations recommendations (12). Within 2 to 3 days, they were also studied by means of dual energy X-ray absorption (DEXA) to assess body composition. DEXA was performed in the Department of Science, Nutrition and Microbiology, Nutrition Section, Università degli Studi di Milano.

Euglycemic-hyperinsulinemic clamp. Subjects were admitted to the Metabolic Unit of the Division of Internal Medicine of the Istituto Scientifico H San Raffaele at 7:00 A.M. after a 10-h period overnight fast. A Teflon catheter was inserted into an antecubital vein for infusions, and an additional one was inserted retrogradely into a wrist vein for blood sampling. The hand was kept in a heated box (50°C) throughout the experiment to allow sampling of arterialized venous blood. A bolus of [6,6-$^2H_2$]glucose (5 mg/kg body wt) followed by a continuous infusion (0.05 mg · kg body wt^{-1} · min^{-1}) obtained from massTrace (Woburn, MA) was administered. Blood samples for postabsorptive plasma glucose, insulin, C-peptide, total proinsulin immunoreactivity (PIM), intact proinsulin, and glucagon were performed in duplicate in the postabsorptive condition. After a 150-min tracer equilibration period, a euglycemic/hyperinsulinemic clamp was performed as previously described (12). Insulin was infused at 40 mU/[m^2 · min] to reach a plasma insulin concentration of 350–400 pmol/l, and plasma glucose concentration was kept at ~5 mmol/l for an additional 150 min by means of a variable infusion of 20% dextrose infusion. Blood samples for plasma hormones, substrates, and tracer enrichment were drawn every 15 min throughout the study.

Indirect calorimetry. Indirect calorimetry was performed continuously for 45 min during the equilibration period and at the end of the clamp as previously described (13).

Oral glucose tolerance test. The OGTT was performed at 8:00 A.M. the day after the insulin clamp following the American Diabetes Association recommendations (12). A Teflon catheter was inserted into an antecubital vein for blood sampling, and samples were obtained for glucose, insulin, C-peptide, PIM, and intact proinsulin in the basal period and after the oral glucose load (75 g) at 30-min intervals for 180 min.

Body composition. DEXA was performed with a Lunar-DPX-IQ scanner (Lunar, Madison, WI) as previously described (14).

Assessment of insulin sensitivity and insulin secretory profile during OGTT in patients with recent onset type 2 diabetes

To compare the alterations of glucose/insulin metabolism of MyD patients during the OGTT with those typical of early stages of type 2 diabetes, eight individuals with fasting plasma glucose >6.1 mmol/l (HbA_{1c}, 7.6%), in which the performance of the OGTT demonstrated the onset of diabetes according to the American Diabetes Association recommendations, were included for deconvolution of OGTT data.

Analytical procedures

Plasma glucose was measured with a Beckman glucose analyzer (13,14). Plasma insulin was measured with a microparticle enzyme immunoassay technology (14) (coefficients of variation [CVs], intra-assay = 4.6%; interassay = 5.4%) (IMx Insulin assay; Abbott Laboratories, Rome, Italy) previously tested for no cross-reactions (15) with proinsulin, C-peptide, and glucagon. C-peptide was measured with a radioimmunoassay using a double-antibody (14) (CVs, intra-assay = 2.3%; interassay = 4.1%). Total PIM was measured by means of highly specific two-site monoclonal antibody-based immunosorbent assay (ELISA; Dako Diagnostics, Cambridgeshire, UK) (14). Intact proinsulin was measured by ELISA with a similar methodological set-up but different antibodies as previously described (ELISA; Dako Diagnostics) (14) (CVs, intra-assay = 4.5%; interassay = 6.8%). Plasma glucagon concentration was assessed as previously described (13) (CVs, intra-assay = 5.5%; interassay = 7.9%). The titer of immunologic markers of type 1 diabetes such as antibodies anti-GAD, -ICA, and -IA2 were measured as previously described (16). The D_2-glucose enrichment was measured by gas chromatography–mass spectrometry as previously described (17). Expansion size in MyD patients was assessed on DNA extracted from peripheral blood samples as previously described (2). All patients had expansion size >150 triplets (min, 150; max, 1,000) for an average of 524 ± 149 triplets.

Calculations

Clamp studies. Glucose kinetic was calculated using Steele's equations for the nonsteady state (18). Endogenous glucose production was calculated by subtracting the glucose infusion rate from the rate of glucose appearance measured with the isotope tracer technique. Total body glucose uptake was determined during the clamp by adding the rate of residual endogenous glucose production to the exogenous glucose infusion rate. Rates of glucose oxidation were calculated from the non-protein respiratory quotient using the tables of Lusk as previously described (13,16). Nonoxidative glucose disposal was calculated by subtracting the glucose oxidation rate from the tissue glucose disposal. Protein oxidation was estimated from urinary nitrogen excretion

Table 2—*Fasting plasma glucose and insulin concentrations in the postabsorptive (basal) and insulin-stimulated states (insulin) and parameters of $SI_{p(clamp)}$*

	MyD			OFF			Control subjects		
	Basal	Insulin	%	Basal	Insulin	%	Basal	Insulin	%
Plasma glucose (mmol/l)	4.59 ± 0.23*	4.56 ± 0.16	—	5.08 ± 0.15	4.89 ± 0.07	—	5.09 ± 0.13	4.98 ± 0.15	—
Plasma insulin (pmol/l)	46 ± 10	407 ± 54	—	52 ± 10	371 ± 22	—	37 ± 5	354 ± 18	—
Plasma C-peptide (nmol/l)	0.49 ± 0.08	0.46 ± 0.11	5 ± 15*	0.46 ± 0.07	0.24 ± 0.10	48 ± 9	0.54 ± 0.09	0.25 ± 0.09	54 ± 11
Plasma glucagon (pg/ml)	94 ± 30	71 ± 24	24 ± 8	93 ± 15	76 ± 18	19 ± 5	102 ± 25	82 ± 30	20 ± 4
Endogenous glucose production (mg/[kg · min])	1.96 ± 0.09	0.38 ± 0.15	82 ± 7	2.30 ± 0.28	0.37 ± 0.16	85 ± 7	2.22 ± 0.11	0.33 ± 0.11	85 ± 5
Glucose disposal (mg/[kg LBM · min])	—	7.22 ± 0.22	—	—	6.32 ± 0.57†	—	—	8.48 ± 0.80	—
Glucose oxidation (mg/[kg LBM · min])	1.54 ± 0.26	3.40 ± 0.55	137 ± 43	1.85 ± 0.45	3.44 ± 0.61	90 ± 35	1.82 ± 0.27	3.35 ± 0.55	91 ± 30
Non-oxidative glucose disposal (mg/[kg LBM · min])	—	3.84 ± 0.84	—	—	2.88 ± 0.85†	—	—	5.13 ± 0.70	—
$SI_{p(clamp)}$ (dl/[min · kg LBM]/(μU/ml) × 10^4)	—	16.5 ± 3.4	—	—	14.3 ± 2.9†	—	—	18.1 ± 2.3	—
QUICKI	0.37 ± 0.01	—	—	0.37 ± 0.01			0.37 ± 0.01	—	—

Data are means ± SEM. *$P < 0.02$ vs. OFF and control subjects; †$P < 0.05$ vs. control subjects.

(19). Quantitative insulin sensitivity check index (QUICKI) was calculated as previously described (20): QUICKI = $1/[\log(I_0) + \log(G_0)]$.

OGTT studies. To characterize the β-cell function, we calculated an index, Φ (μU/ml per mg/dl), reflecting posthepatic insulin delivery rate. Φ was calculated as the ratio between the area under the curve (AUC) of incremental insulin concentration and AUC of incremental glucose concentration. The early insulin response, Φ_{30}, was calculated as the ratio between the AUC of incremental insulin concentration and AUC of incremental glucose concentration during the first 30 min of the oral glucose challenge as recently suggested by Jensen et al. (21).

Statistical analysis

All data are presented as a mean ± SEM. AUC for glucose and hormones during the OGTT were determined by the trapezoidal method. Comparisons between groups were performed using one-way ANOVA and Tukey's post hoc tests. Analysis of the time course of glucose and hormones was performed using the repeated measures ANOVA and Tukey's post hoc tests. Relationships between variables were assessed using the simple correlation analysis.

RESULTS

Anthropometric parameters

Patients with MyD, OFF, and healthy control subjects were carefully matched for sex, age, body weight, height, BMI, waist-to-hip ratio, ideal body weight, and physical activity. Nevertheless, patients with MyD were characterized by altered body composition: they had a marked reduction in lean body mass (LBM, $P < 0.02$; Table 1) and a slight increment of the fat mass that altogether caused a general increment of the percent body fat component ($P < 0.05$), particularly in the arms and trunk ($P < 0.05$; Table 1). The eight individuals in whom type 2 diabetes was diagnosed at the time the OGTT was performed bore the typical anthropometric features of patients with type 2 diabetes (Table 1).

Insulin clamp study

Postabsorptive plasma glucose concentration was reduced in MyD in comparison with OFF and control subjects (Table 2; $P < 0.02$), and the endogenous glucose production was accordingly, but not significantly, reduced. Glucose disposal expressed per kilogram of LBM was comparable in MyD and control subjects (Table 2, $P = 0.22$), as both the glucose oxidative and nonoxidative disposal were similar in the two groups of study (Table 2). On the contrary, OFF proved to be insulin resistant ($P < 0.05$; Table 2) due to a defect in the nonoxidative glucose metabolism ($P < 0.05$; Table 2). Accordingly, the clamp-derived index of insulin sensitivity [$SIp_{(clamp)}$] was also not different between MyD and control subjects ($P = 0.69$) but impaired in OFF ($P < 0.05$). Postabsorptive plasma insulin (Table 2) and C-peptide (Table 2) levels were similar in MyD, OFF, and control subjects. However, plasma PIM ($P < 0.05$) and intact proinsulin ($P < 0.05$) concentrations were increased in MyD in comparison with OFF and control subjects as well as the intact proinsulin/insulin ratio (23 ± 5% vs. 8 ± 1% and 10 ± 2%, respectively; $P < 0.01$). During the insulin clamp, plasma insulin similarly increased in the study groups (Table 2). Insulin-induced suppression of plasma C-peptide ($P < 0.01$), total PIM (0 ± 13 vs. 28 ± 11 and 32 ± 8% in MyD, OFF, and control subjects; $P < 0.01$), and intact proinsulin (0 ± 9 vs. 27 ± 5 and 26 ± 6% in MyD, OFF, and control subjects; $P < 0.01$) concentrations in the last hour of the clamp was impaired in MyD, suggesting a defective feedback autoinhibition of insulin secretion. The insulin-dependent suppression of plasma glucagon concentration (Table 2) was not different in MyD, OFF, and control subjects.

OGTT study

During the oral glucose challenge, the levels of plasma glucose, insulin, and C-peptide were not different in MyD in comparison with OFF and control subjects (Fig. 1). In addition, the index of the insulin secretory response to the oral glucose (Φ: 1.80 ± 0.49, 2.13 ± 0.79, 1.88 ± 0.54 μU/ml per mg/dl; $P = 0.91$) was also comparable in MyD, OFF, and control subjects and decreased only in type 2 diabetic subjects (0.50 ± 0.14; $P = 0.01$). Conversely, the plasma levels of intact proinsulin showed a disproportionate increment in MyD patients in comparison with OFF and control subjects ($AUC_{intact\ proinsulin}$) (Fig. 1). A more careful observation of the hormones' profile during the OGTT suggested a different early response to the oral glucose challenge in MyD patients. In fact, the $AUC_{insulin}$ calculated during the first 30 min of the OGTT were significantly increased in MyD in comparison with OFF and control subjects ($P < 0.05$; Fig. 2) as well as the Φ_{30} ($P < 0.05$; Fig. 2). The C-peptide response during the first 30 min also showed a trend that increased in MyD patients ($P = 0.08$; Fig. 2). Confirming the analysis of data performed in the 3-h interval of time, the analysis of the first 30 min revealed a marked increment of the PIM and intact proinsulin response (Fig. 2; $P < 0.01$).

Overview on insulin sensitivity

The fact that the fasting-derived index of insulin sensitivity (QUICKI; Table 2) and moreover the insulin clamp-derived indices of insulin sensitivity (glucose uptake, oxidative and nonoxidative glucose disposal, $SIp_{(clamp)}$, and the endogenous glucose production as marker of hepatic insulin sensitivity; Table 2) were not different than in control subjects supported the conclusion that in MyD insulin sensitivity was not impaired. This observation is further supported by the relations between QUICKI and $SIp_{(clamp)}$s ($r = 0.70$, $P < 0.0004$). Normal or close-to-normal insulin sensitivity in MyD is further reflected by the comparison with OFF, which as expected showed a typical impairment of insulin action (11,14).

Overview on insulin secretion

The AUC for glucose, insulin, and C-peptide during the OGTT and the OGTT-derived index of insulin secretion, Φ, were comparable in MyD, OFF, and control subjects. Nevertheless, two major secretory dysfunctions characterized MyD patients. First, both in the postabsorptive state and after the OGTT, plasma levels of PIM and intact proinsulin were considerably increased in comparison with OFF and control subjects. Second, MyD patients showed an exaggerated secretory response during the first 30 min after oral glucose administration. Comparison with OFF demonstrated that these alterations were typical of nondiabetic MyD patients and were not a general feature of high-risk individuals (Figs. 1 and 2). In addition, because increased fasting proinsulin levels were described in patients with type 2 diabetes, we also studied a subgroup of eight individuals with diabetes at onset and found that they were characterized by

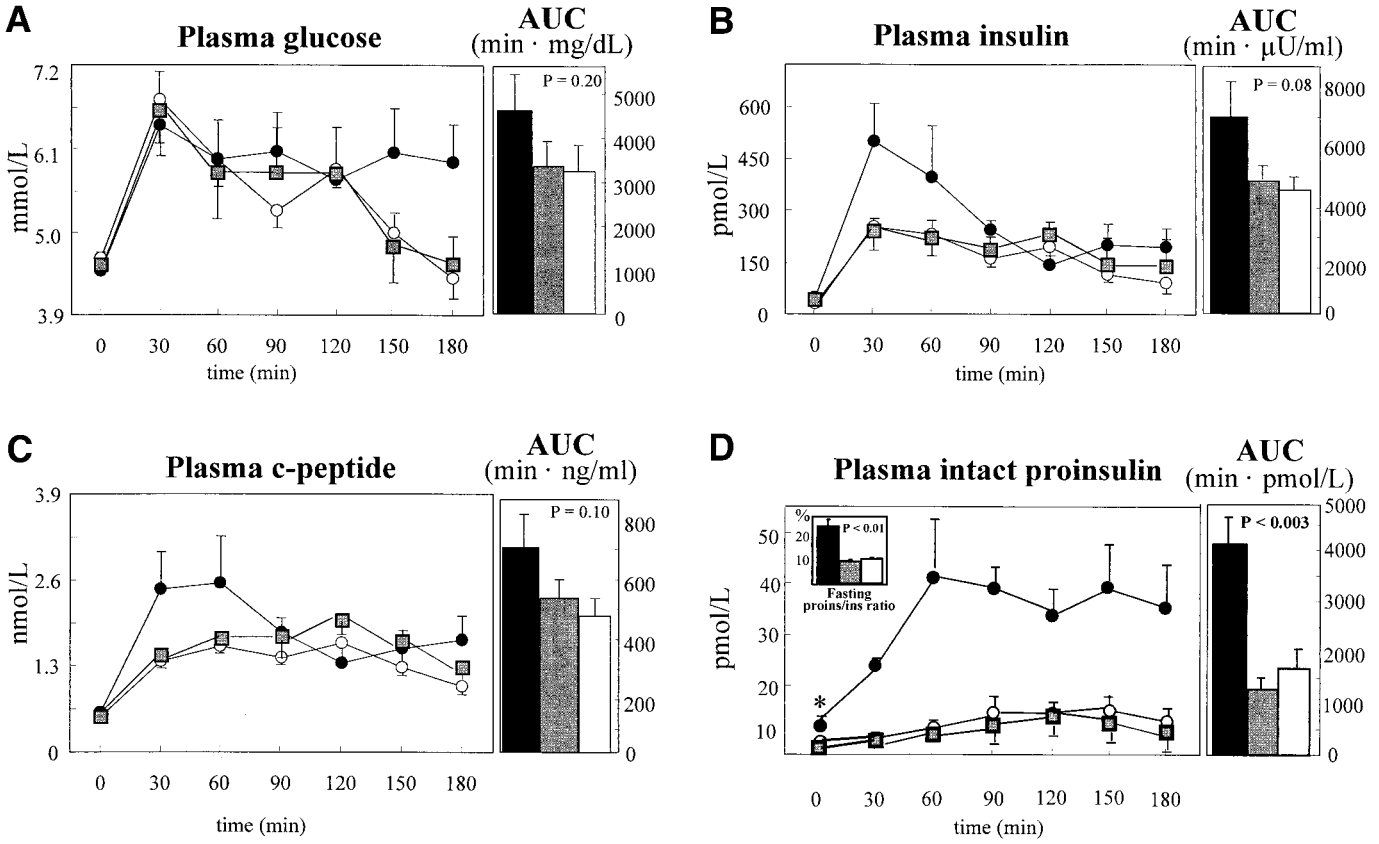

Figure 1—*The time course of glucose* (A)*, insulin* (B)*, C-peptide* (C)*, and intact proinsulin* (D) *concentrations during the OGTT is summarized. Summary of the AUC is also shown in the form of bar graphs besides graphical representation of the time course. The small window in* D *represents the fasting proinsulin/insulin ratio. Black symbols indicate patients with MyD, gray symbols indicate offspring of type 2 diabetic parents, white symbols represent healthy individuals. Statistical analysis was performed using the repeated measures ANOVA and Tukey's post hoc tests.* *$P < 0.05$ *vs. offspring of type 2 diabetic parents and healthy individuals.*

a similar alteration of the postabsorptive plasma proinsulin levels, but during the OGTT the proinsulin response was not similar to that of MyD patients and was considerably reduced.

CONCLUSIONS — The aim of the present work was to identify early alterations of insulin action and secretion in patients with MyD and to define their contribution in conferring a high risk to de velop type 2 diabetes in these individuals. MyD is the most common adult form of muscular dystrophy in which a generalized defect of RNA metabolism has been proposed to be responsible of the clinical manifestations of this disease. Therefore, insulin resistance has been (8,9) claimed as the major metabolic abnormality leading to the development of diabetes in these individuals. This work demonstrated that in young nondiabetic MyD patients insulin resistance was not a dramatic metabolic abnormality and that a major abnormality in these individuals was represented by an alteration of insulin metabolism inducing a considerable increment of plasma proinsulin concentrations in the postabsorptive state, during the insulin challenge (clamp), during the oral glucose challenge (OGTT), and finally by a remarkably higher-than-normal early secretory response after the OGTT. In addition, it was demonstrated that these alterations were peculiar of these high-risk individuals, and that they were not detectable in the typical high-risk population of first-degree relatives of patients with type 2 diabetes.

Because MyD is characterized by progressive muscle wasting (1), we assessed body composition in all subjects by means of DEXA and demonstrated that MyD patients were characterized by a marked reduction of LBM with respect to healthy subjects selected to be comparable for anthropometric parameters. This measurement allowed us to properly normalize the parameters of insulin-dependent metabolism, and we realized that insulin resistance was not as dramatic as expected in our MyD patients because both insulin-stimulated oxidative and nonoxidative glucose metabolism were comparable with control subjects relative to kilograms of LBM. Of other parameters of insulin sensitivity, not derived from the insulin clamp, QUICKI consistently supported the observation of an insulin sensitivity not markedly different than that in control subjects. We speculate that a likely reason for this discrepancy with previous works may be due to the fact that, to our knowledge, this is the first study in which the gold-standard technique to assess insulin sensitivity, the insulin clamp, was performed in MyD combined with the assessment of LBM. In addition, the importance of an overweight or obese condition, in case-control studies, may have been underscored in the past.

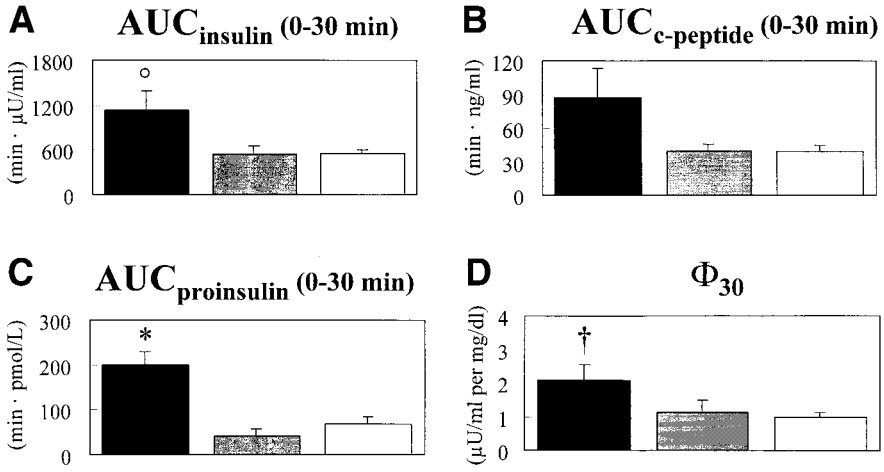

Figure 2—*The AUCs of insulin (A), C-peptide (B), intact proinsulin (C) concentrations during the first 30 min of the OGTT, and Φ_{30} (D) calculated in the same time interval are summarized. Black bars indicate patients with myotonic dystrophy, gray bars indicate offspring of type 2 diabetic parents, white bars represent healthy individuals. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc tests.* *$P < 0.01$ *vs. offspring of type 2 diabetic parents and healthy individuals;* †$P < 0.05$ *vs. healthy individuals;* °$P < 0.05$ *vs. offspring of type 2 diabetic parents and healthy individuals.*

That insulin resistance in MyD patients is not so severe also became evident when we realized that OFF were characterized by a more striking impairment of insulin sensitivity. Therefore, the high prevalence of diabetes in MyD had to be explained by some other alterations. The possibility that diabetes might be associated to an immunological process involving the β-cell, similar to type 1 diabetes, was tested measuring serum titer for antibodies anti-ICA, -GAD, and -IA2. All patients were serum negative and with normal levels of plasma C-peptide excluding that diabetes may have a pathogenesis similar to that of type 1 diabetes.

Therefore, we looked for other markers of increased risk of developing diabetes and found that in MyD patients circulating levels of proinsulin increased enough to reach a proinsulin/insulin ratio more than twofold higher than in control subjects, confirming a previous study that demonstrated that the hyperinsulinemia in MyD is due to a cross-reaction with proinsulin (using less specific insulin assays) rather than a real increase of plasma insulin concentration (22). We also demonstrated a profound impairment of the feedback auto-inhibition of insulin secretion during the insulin clamp. In these conditions exogenous insulin induces inhibition of the β-cell secretion, which is reflected by a drop in plasma C-peptide concentration (23). In this study, we showed that the drop in C-peptide concentration is paralleled by the drop in plasma PIM and intact proinsulin concentration in control subjects, and that MyD patients, on the other hand, failed to show any change in the concentrations of these peptides. In addition, we also tested whether in a condition of stimulation of insulin secretion, rather than inhibition, the increment of these peptides was similar to that of control subjects; in MyD during the OGTT, the increment of plasma insulin and C-peptide was comparable with that of control subjects, meanwhile the increment of PIM and intact proinsulin was disproportionately increased. Because in patients with type 2 diabetes plasma proinsulin-like peptide concentrations are considered to be increased presumably due to a slower rate of conversion or granules' reduced time of residence in the β-cell (24), we also controlled these features in eight patients in whom type 2 diabetes was recently diagnosed and found a similar alteration in the postabsorptive state. To exclude that this common alteration had the same pathogenic event and to demonstrate that hyperproinsulinemia was a primary event in MyD and a secondary event induced by chronic hyperglycemia in type 2 diabetes, we also tested the subgroup of first-degree relatives of parents with type 2 diabetes. We showed that in these high-risk individuals, the proinsulin levels were similar to that of control subjects and considerably lower in comparison with MyD in either the postabsorptive state or the insulin and oral glucose stimulated states. Hyperproinsulinemia is an important feature because it was very recently shown to predict the development of type 2 diabetes over a 27-year period (25).

An additional secretory dysfunction in MyD was observed. Even if a global evaluation of the OGTT showed a similar insulin secretory pattern, MyD patients showed a different behavior in the early phase after the oral glucose administration, reflected by increased parameters of insulin secretion during the first 30 min of the OGTT (Fig. 2). This is another peculiar alteration of MyD because we failed to observe this feature in OFF. In a condition of a more severe impairment of glucose homeostasis as the impaired glucose tolerance, an early reduction, rather than increment, of insulin secretion after oral glucose load was demonstrated using the same parameters in U.S. citizens regardless of ethnicity (21), further suggesting that the β-cell secretory profile in MyD is truly different than the one characterizing other high-risk individuals.

The MyD protein kinase is involved in the modulation of the Ca^{2+} homeostasis in skeletal muscle cells (26), and Ca^{2+} homeostasis is crucial for β-cell secretion events (27); if the alteration of calcium metabolism of the skeletal muscle also affects the β-cell, then the abnormal pattern of insulin secretion may be related to a malfunction of the MyD protein kinase.

Sometimes the mutation size has been found to be associated with the severity of clinical and metabolic alterations in MyD patients (28), but we failed to observe a relationship of the mutation size with the abnormal levels of circulating proinsulin or with Φ_{30} and the AUC for insulin during the first 30 min of the OGTT in our MyD patients. On the other hand, the lack of relationship is not conclusive because it is known that the expansion size may vary from tissue to tissue (29); our determinations were performed in peripheral blood cells, meanwhile β-cell secretory alterations take place in the islet of Langerhans. In addition, we must admit we are not aware of any assessment of the expansion size at the level of the β-cell.

In conclusion, in young and nondiabetic MyD patients, insulin resistance was not the major metabolic alteration associated with high risk to develop type 2 dia-

betes. Abnormalities of insulin secretion represented by increased plasma proinsulin concentrations and a remarkably higher-than-normal early secretory response after the OGTT are most likely major causes of the increased predisposition to develop diabetes in these individuals.

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