Decreased Insulin Sensitivity of Forearm Muscle in Myotonic Dystrophy

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ABSTRACT Previous studies of patients with myotonic dystrophy have demonstrated hyperinsulinism after glucose loading. This hyperinsulinism has been attributed by some investigators to tissue insulin resistance. We have directly studied insulin sensitivity of forearm muscle in patients having such hyperinsulinism. The effect of an intrabrachial arterial insulin infusion (100 μU/kg per min) on glucose uptake was determined in six cases of myotonic dystrophy, six normal subjects, and in seven disease control subjects with myotonia or wasting from other disorders. There was no significant difference in insulin tolerance comparing myotonic dystrophy patients to the normal and disease control groups. Glucose tolerance and basal insulin levels were normal in the myotonic dystrophy patients, but hyperinsulinism occurred after glucose ingestion. After 25 min of intra-arterial insulin, the mean peak muscle glucose uptake in myotonic dystrophy was 2.54 ± 0.54 μmol/min per 100 ml forearm compared to 5.24 ± 0.86 μmol/min per 100 ml for disease controls ($P < 0.05$). Myotonic dystrophy patients showed a peak glucose uptake increment of only 2.6 ± 0.2-fold over basal contrasted with the disease control value of 6.5 ±1.0-fold ($P < 0.02$) and the normal control value of 8.8 ± 1.1-fold ($P < 0.01$). Thus, there was an absolute as well as a relative decrease in muscle insulin sensitivity in myotonic dystrophy patients compared to both control groups. The peak increments in arterio-superficial venous glucose concentration differences after insulin infusion were not significantly different comparing myotonic dystrophy and control groups.

These data suggest that in myotonic dystrophy, there is insulin insensitivity of skeletal muscle.

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INTRODUCTION

Myotonic dystrophy is a multisystemic, autosomal dominant disease (1, 2) frequently associated with normal glucose tolerance and striking hyperinsulinemia after a glucose load (3–12). Recent investigations suggest that the hyperinsulinemia reflects generalized insulin resistance, and abnormal insulin tolerance test results support this conclusion (3, 11). However, other researchers have observed normal insulin tolerance responses (6, 9, 12, 13), and many myotonic dystrophy patients differ distinctly from other diseased patients with hyperinsulinemia, in that their basal insulin levels are normal (4, 9, 14, 15, 16). No abnormalities of endogenous insulin (6, 8, 10, 16) or proinsulin (16) have been reported and a preliminary search for anti-insulin receptor antibody in one patient was unsuccessful (17). One current hypothesis to explain the hyperinsulinemia of myotonic dystrophy is that there is a defect in the plasma membrane of skeletal muscle or other tissues (18). Consistent with this hypothesis are the abnormalities investigators have described in phosphorylation of erythrocyte and skeletal muscle membranes in patients with myotonic dystrophy (18, 19).

We have, therefore, undertaken a study of in vivo tissue insulin sensitivity in patients with myotonic dystrophy. With the forearm technique, resting forearm muscle and adipose tissue glucose uptake have been measured postabsorptively and after intrabrachial arterial infusion of physiologic concentrations of insulin (20–23). Because of a concern for the effect of skeletal muscle wasting on insulin-mediated glucose uptake, we have studied ambulatory patients with only moderate or slight muscle atrophy and have contrasted the results in our myotonic dystrophy patients with comparably wasted neuromuscular disease controls, as well as with normal volunteers.

METHODS

Subjects. Six myotonic dystrophy patients: five males, B, C, D, E, F, and one female, A, and 13 controls were studied. Seven of the control subjects (G, H, I, J, K, L, M) have other neuromuscular diseases, and they have been specifically selected to search for the effects of myotonia, neurogenic muscle atrophy, and type 1 muscle atrophy as isolated variables. These seven patients have been collectively identified as the disease control group. Six normal volunteers complete the control subjects (N, O, P, Q, R, S). Table I presents individual data (age, height, weight, lean body mass) for the myotonic dystrophy patients and controls.

TABLE I

Age, Height, Weight and Muscle Mass in Six Myotonic Muscular Dystrophy, Seven Disease Control, and Six Normal Control Subjects

Patients	Age	Height	Weight	Ideal*	Lean body mass (^{40}K):height	
					Actual value	Percentage expected normal
	yr	*cm*	*kg*	*%*		*%*
Myotonic dystrophy						
A	41	160	44.7	94	0.207	74
B	30	183	52.3	80	0.215	61
C	32	166	62.2	106	0.259	76
D	33	175	81.1	119	0.284	88
E	48	178	74.8	108	0.224	68
F	20	170	50.0	87	0.224	65
Disease controls						
G (AHC)‡	34	182	63.9	95	0.264	77
H (AHC)‡	42	170	82.6	126	0.234	69
I (CNM)§	21	173	54.3	93	0.254	66
J (MYC)‖	41	179	97.7	120		
K (PMY)¶	25	184	87.6	105	0.367	105
L (ALS)**	29	166	45.0	80	0.237	80
M (ALS)**	56	170	56.0	97	0.241	86
Normals						
N	24	182	77.9	104	0.400	113
O	52	175	65.6	102	0.334	103
P	32	178	77.5	108	0.358	104
Q	23	178	65.2	96	0.328	94
R	23	181	71.7	103	0.355	102
S	25	179	65.5	95		

* Ideal body weight was estimated from Metropolitan Life Insurance Tables, 1959.

‡ G and H have chronic idiopathic anterior horn cell disease (AHC).

§ I has a congenital, hereditary centronuclear myopathy (myotubular myopathy) (CNM).

‖ J has a dominantly inherited form of myotonia congenita (MYC).

¶ K has classical paramyotonia congenita (PMY).

** L and M have amyotrophic lateral sclerosis (ALS).

Myotonic dystrophy. All six myotonic dystrophy patients were ambulatory, and had only slight or moderate forearm muscle wasting. All had myotonia on grip, percussion, and electromyographic testing. Patients A, B, C, D, and E were biopsied and had muscle biopsies characteristic of myotonic muscular dystrophy with type 1 atrophy (24). All patients had a positive family history for this disorder, as well as cataracts and other stigmata of the disease. Four kindreds were studied. Patients E and A are brother and sister. Patients D and C are brothers, whereas patients B and F are the only patients studied from their kindreds.

Normal controls. Patients N, Q, R, and S are asymptomatic, normal weight students. Patients O and P are asymptomatic, normal weight businessmen. Each had normal neurologic and physical examinations and had no history of chronic medical illness. There was no positive family history for metabolic or neurologic diseases.

Disease controls. Patients G, H, I, L, and M are subjects with forearm muscle wasting comparable to that observed in the six myotonic dystrophy patients. Patient G is an ambulatory patient with a 10-yr history of a slowly progressive, idiopathic anterior horn cell disease characterized by weakness, fasciculations, muscle wasting, and depressed reflexes. He has no upper motor neuron signs. Electromyographic evaluation and muscle biopsy demonstrated findings typical of denervation.

Patient H is an ambulatory patient having a history of proximal greater than distal weakness of a very slowly progressive nature for the past 26 yr, diagnosed as Kugelberg-Welander type of spinal muscular atrophy. He is areflexic and has no upper motor neuron signs. Electrodiagnostic evaluation showed diffuse chronic denervation.

Patient M has amyotrophic lateral sclerosis and ambulates only with active assistance as a result leg spasticity. For 12 mo, he has had generalized fasciculations and diffuse weakness with his legs being more affected than his arms. He is dysarthric and has generalized hyper-reflexia and bilateral extensor plantar responses. Myelography was normal; electromyography revealed diffuse acute and chronic denervation.

Patient L also has amyotrophic lateral sclerosis. He has a 3-yr history of diffuse weakness, fasciculations, and dysarthric speech. He ambulates with assistance and has moderate generalized wasting, weakness, and diffuse hyperreflexia with bilateral plantar extensor responses. His myelography was normal; electromyography showed acute and chronic denervation.

Patient I has muscle weakness, wasting, and hyporeflexia more severe distally than proximally. No other neurologic abnormalities are present. Muscle biopsy was characteristic of centronuclear myopathy (myotubular myopathy) with moderate type 1 atrophy (25, 26).

Patients J and K are myotonic control subjects with normal muscle strength and bulk. Patient J has myotonia congenita; he has grip and percussion myotonia but normal strength. The family history is consistent with an autosomal dominant inheritance pattern with both a parent and son similarly affected. Patient K has paramyotonia congenita and normal muscle bulk and strength. The clinical findings have been reported elsewhere (27).

All patients except L and M could easily perform 15 min of continuous hand grip exercise on an exercise ergometer (at a work rate of 7.5 kg-m/min). None of the myotonic dystrophy patients nor any of the control subjects received any medication for 2 wk before evaluation with the exception of the patient J who had taken quinine 300 mg, one to three times a day until 3 days before evaluation.

Total body potassium (^{40}K) was determined with the use of a whole body scintillation counter using sodium iodide crystal to count the gamma ray activity and used to estimate lean body mass as described (28–30).

Each subject was informed of the nature, purpose, and possible risks involved in these studies before giving their consent to participate.

Standard statistical analytic techniques have been used (31). All group data is presented as the mean±SE of the mean.

Experimental protocols. Each study participant was hospitalized in the Strong Memorial Hospital Clinical Research Center, Rochester, N. Y., 3 days before oral gluose and intravenous insulin tolerance testing and before forearm investigation. These investigations were performed on separate admissions 2–4 mo apart. No change in clinical examination nor the parameters listed in Table I were noted on comparison of the two admissions. Each patient was placed on a constant diet consisting of 1–1.5 g/kg per day of protein; 286±15 g of carbohydrate with the remaining portion of the diet composed of fat combined to give a total caloric intake of 32–35 calories/kg per day; 0.02–0.04 g of calcium/kg per day; 40–90 mg potassium/kg per day; and 45–80 mg sodium/kg per day. All participants were allowed unlimited ambulation but no vigorous exercise was undertaken during the 3-day hospital stay. Patients were fasted and at bed rest overnight before each test and recumbent during each test.

Glucose tolerance testing. Each subject was given 1.5 g of glucose per kg (maximum of 100 g) in 200 ml of water, ingested over a period of 5 min. Plasma concentrations of glucose and insulin were determined at 0, 30, 60, 90, 120, 180, 240, and 300 min after starting glucose consumption.

Intravenous insulin tolerance testing. A dose of 0.1-U/kg of pork crystalline zinc insulin was rapidly given as a bolus. An initial sample was drawn to measure plasma glucose and insulin levels immediately before the insulin injection and subsequent samples were collected at 15, 30, 45, 60, 90, and 120 min.

Forearm investigation. Placement of venous and arterial catheters were carried out after an overnight fast. The technique of establishing the blood sample collecting lines, as well as that used to determine blood flow have been described (32). Each study was performed between 7:30 a.m. and 1:30 p.m. with subject supine.

Control (basal) measurements. Three sets of simultaneous blood samples were collected from the artery, deep vein, and superficial vein in heparinized syringes at intervals of 20 min in all subjects. These three sets of samples constituted the control. The metabolism of forearm tissues (mainly muscle) was estimated by the use of the Fick expression $Q_m = F(A\text{-}DV)$ (20–22, 32). A and DV are the arterial and deep venous concentrations for a specific metabolite. F is 85% of total forearm blood flow and is expressed as milliliter per minute per 100 ml of forearm volume. Q_m is the estimated net metabolite flux across forearm muscle.

Insulin infusion. After the control period, simultaneous specimens were drawn at 25, 45, 65, 85, and 105 min after beginning the brachial intra-arterial insulin infusion. Insulin was administered for 25 min at a rate of 100 μU/kg per min[1] (20–22). Insulin infused at this rate produced concentrations of insulin in the high physiologic range within the blood circulating through the forearm (Table II). Unlike the other patients, patient L received the insulin infusion without inflation of a sphygmomanometer about the wrist. To decrease insulin binding to the infusion tubing, salt-poor normal human serum albumin (Albumisol 25%; Merck, Sharp & Dohme Canada Ltd., Montreal, Canada) was added to the Evans blue dye infusates. The final concentration of albumin for patients B, C, E, and controls G, Q, R, and S was 0.12 g/100 ml and for all other patients was 0.25 g/100 ml.

Analyses. Portions of heparinized blood from each collection during the oral glucose tolerance test and intravenous insulin tolerance test were delivered into oxalate-fluoride tubes and plasma glucose was determined in duplicate with a Beckman automated glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.). Plasma from the remaining blood sample was separated for duplicate insulin determination. Insulin concentration was determined with a modification (33) of a double antibody technique (34). Somogyi-Nelson filtrates of whole blood were analyzed for glucose in triplicate using the orthodianisidine-glucose oxidase methodology for all forearm blood samples (35). Insulin was assayed as noted above. Growth hormone was determined with a modification of a described double antibody technique (36). The insulin analysis procedure had a 13.3% intra-assay and a 17.4% interassay variation. The growth hormone analysis had a 6.2% intra assay variation and a 20.9% interassay variation. Additional plasma was used to determine Evans blue dye concentration. Each arterial sample had a hematocrit determination in triplicate with Wintrobe tubes centrifuged at 4,000 g for 20 min.

RESULTS

Oral glucose tolerance test (Tables III and IV). Before glucose ingestion, the myotonic dystrophy patients had basal glucose and insulin levels comparable to the controls. After the glucose load, no remarkable difference in plasma glucose values between myotonic dystrophy patients and normals was noted. The plasma insulin levels for the myotonic dystrophy group were significantly above normal as indicated in Table III. This pattern of excessive insulin release is similar to that reported by other investigators (3–12).

Patients I and M in the disease control group had mild glucose intolerance and were considered latent diabetics (37). Glucose concentration was significantly higher than normal for disease controls at 120 min ($P < 0.05$) but statistical significance was lost with the omission of patient M from the data.

Intravenous insulin tolerance test. Plasma glucose levels before intravenous insulin administration were not significantly different between the myotonic dystrophy patients (4.61±0.2 mM/liter), disease controls (4.66±0.1 mM/liter), and normals (4.44±0.1 mM/liter). The nadir for glucose levels occurred at 30 min for all subjects except normal control, Q, whose maximum decline came at 15 min. Nadir level in plasma glucose was similar for each group; myotonic dystrophy (2.22±0.2 mM/liter), disease controls (2.39±0.2 mM/liter), and normals (2.05±0.2 mM/liter). Four myotonic dystrophy patients' nadir levels were <50% of the fasting glucose. The remaining two had nadir values of 56 and 64% of the basal glucose. Normal controls values fell by >50% of basal except for patients R and O whose

[1] Purified single component regular pork insulin, lot CT-1902-4H, was kindly provided by Dr. John Galloway, Eli Lilly & Co., Indianapolis, Ind.

TABLE II

Effect of Intrabrachial Arterial Insulin (100 μU/kg per min) on Arteriovenous Concentration Difference of Glucose Across Forearm Muscle in Six Myotonic Dystrophy, Seven Disease Control, and Six Normal Subjects

	Deep venous insulin level	Arteriovenous concentration difference of glucose					
After start of insulin, *min*		0	25	45	65	85	105
	μU/ml	*mmol/liter*					
Myotonic dystrophy patients							
E*	70	0.16	0.23	0.47	0.36	0.09	0.28
A	96	0.09	0.20	0.45	0.25	0.41	
C	120	0.29	0.35	0.70	0.72	0.59	0.51
B	123	0.16	0.26	0.44	0.38	0.18	0.00
D	165	0.29	0.44	0.08	0.29	0.39	0.43
F	234	0.29	0.33	0.61	0.36	0.20	0.14
Mean±SEM‡	135±24	0.21±0.04	0.30±0.04	0.46±0.09	0.39±0.07	0.31±0.08	0.27±0.09
Disease Controls							
L (ALS)	46	0.06	0.07	0.26	0.14	0.15	0.19
G (AHC)	128	0.18	0.47	1.64	0.80	0.34	0.41
J (MYC)	171	0.22	1.79	2.37	1.73	1.24	
M (ALS)	189	0.28	0.60	0.82	0.51	0.21	0.46
K (PMY)	194	0.17	0.71	0.69	0.60	0.49	0.21
I (CNM)	225	0.21	0.47	1.02	1.00	0.81	0.73
H (AHC)	260	0.24	0.47	0.55	0.68	0.60	0.32
Mean±SEM	173±26	0.19±0.03	0.65±0.20	1.05±0.27	0.78±0.19	0.55±0.14	0.39±0.08
Normal Controls							
Q	30	0.16	0.60	0.36	0.04	0.07	0.18
R	81	0.08	0.38	0.36	0.21	0.09	0.03
O	108	0.18	0.46	0.84	0.96	0.49	0.37
P	121	0.12	0.97	0.27	0.15	0.18	0.21
N	124	0.11	0.67	0.73	0.57	0.50	0.32
S	170	0.17	1.52	0.73	0.54		0.25
Mean±SEM	106±19	0.14±0.02	0.77±0.17	0.55±0.10	0.41±0.14	0.27±0.10	0.23±0.05
Controls (previously published)§							
Mean±SEM	169±24	0.17±0.04	1.22±0.18	1.27±0.20	1.01±0.12	0.55±0.14	

See Table I for explanation of abbreviations.

* Patients E and Q had dual brachial arterial supplies to their forearms.

‡ Statistical data is presented as mean±SE of the mean.

§ Pozefsky et al. (22), mean peak deep venous insulin and arterio concentration difference of glucose values were calculated from data on seven normal weight young men.

nadir values were 61 and 56% of their fasting glucose levels. Two disease control subjects' glucose levels did not decline by >50% of basal; patient L, 52%, and patient M, 65%. The hypoglycemic response to insulin for the myotonic dystrophy and the control group was similar to the responses described by Roth and associates (38) in normals with this dose of insulin.

Forearm studies

Blood flow determinations. Blood flow values during the control period and after intra-arterial insulin infusion were similar for the myotonic dystrophy, disease controls, and normal control groups (Table V). Paired analysis of blood flow in each subject comparing his resting blood flow to all values after insulin infusion indicated a slight but significant ($P < 0.05$) decline in flow in both disease control (85±7%) and normal control (79±5%) groups after the infusion. This decline occurred at either the 65- or 85-min time point. The fall in blood flow after insulin for the myotonic dystrophy patients was 85±16% and not significant. Paired analysis of peak flow values after in-

TABLE III

Plasma Insulin and Glucose Concentrations in Six Myotonic Dystrophy and Seven Disease Control Patients

	Insulin								Glucose							
Time, *min*	0	30	60	90	120	180	240	300	0	30	60	90	120	180	240	300
	μU/ml								*mM/liter*							
Myotonic dystrophy patients																
A	6	50	76	60	73	11	10	7	4.38	5.94	6.99	7.88	6.99	4.33	3.83	4.27
B	10	133	113	106	64	20	10	10	4.22	9.77	8.49	8.66	7.33	3.27	4.22	4.49
C	13	239	219	274	129	34	23	14	4.99	9.99	10.00	8.71	6.38	5.72	5.55	5.11
D	11	146	113	126	71	33	13	14	4.66	7.10	7.38	7.33	7.05	5.55	5.11	4.73
E	14	165	111	117	162	61	14	26	5.05	6.99	6.60	7.10	7.16	5.72	4.72	4.66
F	13	141	98	45	136	45	22	10	4.49	8.32	6.49	5.38	6.60	5.77	4.44	4.77
Mean±SEM*	11±1	145±25‖	122±20¶	121±33**	106±17‡‡	34±7	15±2	13±3	4.61 ±0.1	8.05 ±0.9	7.66 ±0.6	7.49 ±0.5	6.94 ±0.2	5.05 ±0.4	4.66 ±0.3	4.66 ±0.1
Disease Controls‡																
G	12	69	56	100	74	23	18	9	4.38	8.10	7.88	9.16	7.05	4.33	4.33	4.66
H	14	104	87	77	57	42	15	11	5.16	9.05	8.99	8.38	6.88	6.27	3.72	4.33
I	5	25	31	23	26	12	16	3	4.27	8.71	10.49	9.49	9.43	7.16	3.50	3.83
J	16	98	33	60	56	23	16	11	4.33	9.93	7.60	7.71	7.05	4.73	4.50	5.27
K	11	63	60	36	50	27	14	11	4.61	8.43	8.82	6.44	6.55	5.77	3.61	4.44
L	11	36	50	57	38	28	9	9	5.11	8.10	9.43	8.21	7.44	6.66	4.00	4.83
M	9	77	54	55	56	17	14	11	4.94	9.82	12.15	11.27	12.15	6.99	4.16	3.94
Mean±SEM	11±1	67±11	53±7	58±10	51±6	25±5	15±1	9±1	4.66 ±0.2	8.88 ±0.3	9.32 ±0.6	8.66 ±0.6	8.10 ±0.8	5.98 ±0.4	4.00 ±0.2	4.49 ±0.2
Normal Controls§																
Mean±SEM	10±2	58±7	64±11	53±8	36±4	23±4	10±2	8±2	4.66 ±0.2	8.44 ±0.5	8.21 ±0.7	7.27 ±0.4	6.22 ±0.3	5.27 ±0.3	4.38 ±0.3	4.94 ±0.2

* Statistical data is presented as the mean±SE of the mean.

‡ See Table I for specific diagnoses.

§ These data include the normal controls in Table I plus two normal males and one female, ages 29, 25, and 24 respectively.

‖ $P < 0.01$ compared to normal controls.

¶ $P < 0.02$ compared to normal controls.

** $P < 0.05$ compared to normal controls.

‡‡ $P < 0.001$ compared to normal controls.

TABLE IV

Peak Increment in Forearm Muscle Glucose Uptake above Basal after Intrabrachial Arterial Insulin Infusion (100 μU/kg per min) in Five Myotonic Dystrophy Seven Disease Control and Five Normal Subjects

Patients	Peak deep venous insulin level	Basal muscle glucose uptake forearm	Peak muscle glucose uptake forearm	Fold increase over basal uptake	*Cumulative insulin increments after glucose ingestion
	μU/ml	μM/min/100 ml	μM/min/100 ml		μU/ml
Myotonic dystrophy					
A	96	0.57	1.53	2.7	245
B	120	1.18	3.71	3.1	841
C	123	0.76	1.59	2.1	388
D	165	0.94	1.87	2.0	437
E	234	1.36	3.99	2.9	406
‡Mean±SEM	148±24	0.96±0.14	2.54±0.54	2.6±0.2	463±99
Disease§ Controls					
L (ALS)	46	0.74	2.62	3.6	150
G (AHC)	128	0.97	8.77	9.0	268
J (MYC)	171	0.65	6.89	10.6	185
M (ALS)	189	0.90	2.90	3.2	221
K (PMY)	194	0.70	3.71	5.3	184
I (CNM)	225	1.05	6.18	5.9	136
H (AHC)	260	0.72	5.64	7.8	295
Mean±SEM	173±26	0.82±0.06	5.24±0.86	6.5±1.0	206±22
Normal Controls					
R	81	0.20	1.25	6.2	147
O	108	0.90	5.54	5.6	171
P	121	0.44	5.40	12.3	210
N	124	0.65	4.75	7.3	122
S	170	0.61	5.05	8.3	185
Mean±SEM	121±14	0.56±0.12	4.04±0.80	8.8±1.1	170±13
Controls (previously published)					
Pozefsky‖	169±24	0.50±0.1	5.05±1.2	10.1	
Andres et al.¶	200–500	0.59	6.78	11.5	

* Data calculated by subtracting basal insulin concentration from each insulin level measured during 5 h glucose tolerance test and adding all these increments. The cumulative values for the myotonic dystrophy patient, E, and the normal control, Q, (whose blood flow determinations were prevented by dual brachial arteries), were 558 and 184 μU/ml respectively.

‡ Statistical data is presented as mean±SE of the mean.

§ See Table I for explanation of abbrevations.

‖ Pozefsky et al. (22), calculated mean±SEM data from seven normal weight young men.

¶ Andres et al. (39), forearm experimental data calculated from six men and four women all normal weight young adults.

sulin infusion revealed a slight but significant ($P < 0.05$) rise in the disease control (23±7%) and normal control (30±10%) patients, which came in each subject either 25 or 45 min after infusion. Despite these transient changes in flow, there was generally stable blood flow for each individual throughout the study. Similarly, blood pressure and heart rate were virtually unchanged throughout each subject's forearm study. The 65-, 85-, and 105-min blood flows for the myotonic dystrophy patient, F, were omitted due to poor agreement between deep and superficial Evans blue dye concentrations (20).

Insulin infusion. Deep venous insulin levels achieved during infusion are noted in Table II. Deep venous insulin levels were generally lower for the myotonic dystrophy group compared to the disease

TABLE V

Forearm Blood Flow before, during and after Intrabrachial Arterial Insulin Infusion (100 μU/kg per min) in Five Myotic Dystrophy Patients, Seven Disease Controls and Five Normal Volunteers

	Blood Flow*					
Time after starting insulin, *min*	Control	25	45	65	85	105
Patients						
Myotonic dystrophy	5.26±0.6	5.47±0.3	5.07±0.9	4.53±0.7	4.04±0.2	4.30±0.1
Disease controls	5.12±0.6	5.43±0.5	6.04±1.1	4.74±0.6	5.80±0.9	5.68±0.5
Normals	4.70±0.5	6.22±0.9	5.27±0.7	4.10±0.5	4.28±0.6	5.40±0.3

* Blood flow calculation was not possible in patients E and Q (Table I) as a result of a dual arterial supply.

control group and higher when compared to the normal subjects, but these differences were not statistically significant.

Only slight changes in arterial insulin occurred during the studies. Before intra-arterial insulin infusion, basal arterial insulin levels were similar for the myotonic dystrophy (11±2 μU/ml), disease control (9±2 μU/ml), and normal controls (9±2 μU/ml). Arterial insulin values at the end of insulin infusion (25 min point) rose showing an increment above basal of 10±6 μU/ml for myotonic dystrophy, 6±2 μU/ml for disease control, and 8±5 μU/ml for normal control. Paired analysis combining myotonic dystrophy and all control subjects showed a slight, significant increment above basal arterial insulin at 25 min (8±2 μU/ml, $P < 0.01$). The 45-, 65-, 85-, and 105-min arterial and deep venous insulin levels were not significantly different from basal values in either the myotonic dystrophy or control groups.

Insulin-stimulated muscle glucose uptake. Basal arteriodeepvenous glucose concentration differences were similar for the myotonic dystrophy and control subjects and comparable to published data by Pozefsky and co-workers (21, 22) (Table II). After intra-arterial insulin infusion, however, consistently lower increments in arteriodeepvenous concentration differences over basal were observed in the myotonic dystrophy group compared to the disease control and normal control groups. Comparison of the mean peak arteriodeepvenous glucose concentration difference of the myotonic dystrophy patients (patients A, B, C, D, F) to that observed in four comparably wasted disease controls (patients G, H, I, M) reveals a significantly lower peak value, 0.53±0.06 mM/liter versus 1.04 ±0.21 mM/liter, $P < 0.05$. A similar comparison of these five myotonic dystrophy patients to four normal controls (patients N, O, P, and S) reveals significantly lower peak values in this myotonic dystrophy group, 0.53±0.06 mM/liter vs. 1.05±0.17 mM/liter, $P < 0.02$.

Arteriodeepvenous concentration differences vary with blood flow and a more accurate comparison of insulin-mediated muscle glucose uptake between the myotonic dystrophy group and controls can be made by examining the net glucose flux into muscle. Table IV contrasts the basal and peak insulin-stimulated muscle glucose uptake between the myotonic dystrophy and control groups. Further comparison with published normal studies is included (22, 39). Basal muscle glucose uptakes are similar for both the myotonic dystrophy and disease control groups with the normal controls being somewhat lower. Subjects with mild to moderate muscle wasting (patients A, B, C, D, E, F, G, H, I, L, M) have higher resting glucose uptake compared to those with normal muscle bulk. Such a comparison reveals basal glucose uptake for muscle wasted subjects of 0.92±0.1 μmol/min per 100 ml forearm vs. 0.59±0.1 μmol/min per 100 ml for the nonwasted subjects ($P < 0.02$). No significant difference in basal glucose uptake was apparent comparing the myotonic dystrophy to wasted disease control patients. After intrabrachial arterial insulin infusion, the mean peak muscle glucose uptake in the myotonic dystrophy group was lower than in disease control patients. This diminished insulin-stimulated muscle glucose uptake in the myotonic dystrophy patients was emphasized by examining the maximum increase in muscle glucose uptake after insulin over basal value (Table IV).

Comparison of the four wasted disease control patients (G, H, I, and M) to the five myotonic dystrophy patients (A, B, C, D, F) showed significantly higher mean peak muscle glucose uptake in these disease controls and a higher fold increase over basal. However, one wasted disease control, M, with amyotrophic lateral sclerosis, showed a decreased peak muscle glucose uptake and a low fold increase over basal that were indistinguishable from the myotonic dystrophy group. This patient was the only subject studied demonstrating definite glucose intolerance (Table III) and also the smallest decline in plasma glucose after intravenous insulin (5.11 mmol/liter to 3.33 mmol/liter, 65%). The insulin resistance noted during

oral glucose tolerance and intravenous insulin tolerance testing were supported by the decreased response to insulin seen in the forearm study data.

There was no correlation between the cumulative insulin release during oral glucose tolerance testing and the increase in forearm muscle glucose uptake after insulin infusion in the myotonic dystrophy patients (Table IV).

Growth hormone levels during forearm studies. The level of arterial growth hormone with all the individual arterial values for the myotonic dystrophy group was 2.8±0.7 ng/ml compared to 3.9±0.7 ng/ml for the disease control group, and 3.3±0.3 ng/ml for normal controls. There was no significant difference between the groups.

Insulin-stimulated glucose uptake by superficial tissues. Arteriosuperficial venous (skin and fat) glucose concentration differences were examined to estimate the insulin sensitivity of adipose tissue. Technical difficulties (low skin blood flow) prevented adequate blood collections at several time points in two myotonic dystrophy patients. 45-, 65-, 85-, and 105-min samples were insufficient for patients E and C. Only one normal control subject, S, and one disease control, M, had similar sample collection difficulty, and the 45- to 105-min samples were not adequate. Despite these problems, certain trends were noted. The mean basal arteriosuperficial venous glucose concentration difference was 0.31±0.04 mmol/liter in the myotonic dystrophy patients compared to 0.31±0.04 mmol/liter for disease controls, and 0.26±0.03 mmol/liter in the normal control group. After insulin infusion, the mean peak increment in arteriosuperficial venous concentration difference over basal was 0.27±0.12 mmol/liter in the myotonic dystrophy group compared with a disease control group value of 0.30±0.05 mmol/liter, and 0.38±0.14 mmol/liter in normal controls. These values were not significantly different and are comparable to grouped data reported for normals (21).

DISCUSSION

Oral glucose tolerance testing has demonstrated normal glucose tolerance and normal basal insulin levels in our six myotonic dystrophy patients with similar findings observed in the disease control subjects except for M and I who showed mild glucose intolerance. These findings are comparable to many cases of myotonic dystrophy described by other investigators (3–12). After glucose ingestion, an excessive insulin release occurred in the myotonic dystrophy group with greater than control levels maintained for the first 2 h of the test. This hyperinsulinism was expected, having been well documented in the past (3–12). Unlike the hyperinsulinism seen in obese individuals (40), our myotonic dystrophy group had excessive insulin release after a glucose load despite presence of normal basal levels.

Intravenous insulin tolerance testing revealed a normal maximum fall in blood glucose for the myotonic dystrophy patients compared to our disease control and normal control groups and compared to published normal data (38). Two studies of myotonic dystrophy have shown their patients to have less than the expected fall in blood glucose (3, 11) after intravenous insulin whereas other investigations have reported a normal response (6, 9, 12, 13). A more recent study using frequent glucose sampling after intravenous insulin injection has reported a significantly higher mean nadir value and a slower rate of fall of plasma glucose in 14 myotonic dystrophy patients compared to normals (11). The insulin resistance observed in each patient correlated well with the degree of hyperinsulinism seen in the various provocative tests that they received. Our results suggest a normal total body responsiveness to intravenous insulin in our myotonic dystrophy group. More frequent glucose sampling might, however, have revealed a mild degree of insulin resistance.

The forearm study results have shown a decreased insulin-stimulated glucose uptake of forearm skeletal muscle in patients with myotonic dystrophy. Intra-arterial infusion of physiologic concentrations of insulin for 25 min resulted in an eightfold increase in glucose uptake in the normal control subjects, a sixfold increase in disease controls, but only a two- to threefold increase in the myotonic dystrophy group. This diminished insulin-stimulated uptake was both absolute as well as relative. Decreased uptake of glucose appeared to be selective for skeletal muscle because a normal peak increase in arteriovenous concentration difference of glucose occurred in superficial tissues (largely skin and adipose tissue).

The basis for the apparent insulin resistance of forearm skeletal muscle in patients with myotonic dystrophy is unclear. The possibility that this insulin insensitivity might be a nonspecific reflection of muscle atrophy was addressed by studying wasted neuromuscular disease control patients (G, H, I, L, M) with a comparable degree of forearm wasting and weakness.

The wasted disease control, M, with amyotrophic lateral sclerosis demonstrated an insulin-stimulated forearm muscle glucose uptake indistinguishable from that noted for the myotonic dystrophy group. Patient M is the only wasted disease control with a clearly diminished insulin responsiveness compared to the other three patients with lower motor neuron disease (G, H, L) and compared to the patient with primary myopathy I. This abnormality may be unrelated to his anterior horn cell disease as the present data

suggests, but additional studies of patients with lower motor neuron diseases are needed to verify this impression.

Because myotonic dystrophy often has atrophy affecting type 1 muscle fibers to a greater extent than type 2 (24), special effort was made to study a patient with another primary myopathy with wasting of type 1 fibers. Wasted disease control, I, had marked type 1 atrophy as a result of centronuclear myopathy, and did not show insulin insensitivity.

Type 1 muscle fibers usually resemble the red (slow twitch) muscle fibers whereas type 2 fibers resemble the white (fast twitch) muscle described in animal studies (41–43). Red muscle has been shown to have a greater basal glucose uptake than white muscle when incubated in vitro in the absence of insulin (44). Other investigations comparing glucose uptake in the rat diaphragm (60:40, red:white) (45) to the gastrocnemius (mainly white) in the same animal have shown a two- to threefold greater insulin-stimulated glucose uptake per gram of muscle by the diaphragm over the gastrocnemius (46). These findings suggest that type 1 fiber atrophy might diminish peak insulin-stimulated muscle glucose uptake. The myotonic dystrophy patients, however, show diminished increases in muscle glucose uptake compared to patient I.

The possibility that the myotonic phenomenon was related to the insulin insensitivity in the myotonic dystrophy patients was also examined. Two control patients (J and K) with severe myotonia, one with myotonia congenita, and one with paramyotonia congenita were investigated. Both had normal insulin-stimulated glucose uptake.

Another explanation for the decreased insulin-stimulated muscle glucose uptake in myotonic dystrophy might propose that a lower than normal fraction of total forearm blood flow goes to muscle. If this were the case and the myotonic dystrophy patients were felt to have normal muscle insulin sensitivity, the arteriodeepvenous glucose concentration differences should have been normal or greater than normal. Although we have not used isotopic methods for separate measurement of skin and muscle blood flow, it seems likely that at least a normal fraction of flow has gone to forearm muscle in the myotonic dystrophy patients. During all our subjects' forearm studies, the collection of blood samples from the deep venous line was subjectively much easier than those from the superficial venous line. Thus, there were no gross qualitative differences noted in the distribution of blood flow among all our patients.

The possibility that the insulin insensitivity is related to growth hormone excess was examined. Normal levels of growth hormone were found throughout the forearm studies. The reported hyperresponsiveness of patients with myotonic dystrophy to growth hormone (47) could contribute to the muscle insulin insensitivity which we have observed. A more recent report suggests that this hyperresponsiveness to growth hormone occurs only in males with myotonic dystrophy (48). Because the female patient which we studied was also insulin resistant, the hyperresponsiveness hypothesis does not account for the observed insensitivity of her skeletal muscle to insulin.

Previous forearm insulin infusion studies indicate that normal subcutaneous fat has a lower sensitivity to insulin-stimulated glucose uptake than muscle (20–22). If the myotonic dystrophy patients were to have large fat deposits in their forearm muscle compared to other disorders causing wasting, this might produce diminished insulin stimulated muscle glucose uptake. The muscle biopsies taken in our myotonic dystrophy patients show remarkably little accumulation of perifascicular or intramuscular fat. An increase in muscle adipose tissue, though possible, seems an unlikely explanation for the insulin insensitivity seen in our myotonic dystrophy group.

If all or a portion of the skeletal muscle in our myotonic dystrophy patients possessed insulin resistance while other tissues maintained normal sensitivity, we could explain the paradoxical combination of normal intravenous insulin tolerance and decreased insulin mediated muscle glucose uptake. Such a hypothesis might also explain the normal glucose tolerance. Other investigators have reported that a large part of an oral glucose load is removed by the liver and that peripheral muscle may incorporate no >15% of the load (49). If the liver is normally insulin sensitive along with subcutaneous tissue in our myotonic dystrophy group, the major fraction of an ingested glucose meal would be cleared normally.

Another hypothesis to explain the paradoxical normal response to intravenous insulin injection uses the possibility that a critical plasma concentration of insulin may be needed to facilitate muscle glucose uptake. The insulin levels during an intravenous insulin injection, oral glucose tolerance test, or tolbutamide tolerance test may exceed this critical level in myotonic dystrophy and thereby lead to normal tissue glucose uptake. Subsequent investigations are required to test this hypothesis and the one offered in the preceding paragraph. Such future studies will clarify the alteration that has produced the insulin insensitivity in patients with myotonic dystrophy.

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