Carbohydrate metabolism in motor neurone disease

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SUMMARY Eight patients with classical motor neurone disease, and eight control patients with neurological disease and matched for degree of wasting, were studied. Both groups had abnormal glucose tolerance, but the patients with motor neurone disease had a significantly lower insulin response both to oral glucose loading and to intravenous tolbutamide. These results suggest that in patients with motor neurone disease there is an impaired synthesis or release of insulin due to islet cell damage. Blood pyruvate and lactate, and cerebrospinal fluid pyruvate, lactate, and citrate, did not differ significantly from the control group. Blood citrate levels were significantly higher in patients with motor neurone disease compared with the controls. Triglyceride levels were raised in patients with motor neurone disease compared to the control patients. This may be secondary to the increased citrate levels.

A high incidence of abnormal glucose tolerance to both oral and intravenous testing has been reported in patients with motor neurone disease (Matthews, 1958; Ionasescu and Luca, 1964; Steinke and Tyler, 1964; Quick and Greer, 1967; Collis and Engel, 1968). The pathophysiological mechanism for the abnormal response is still controversial. Gotoh *et al.* (1972) showed blunted insulin levels in response to oral glucose loads, and the response to intravenous tolbutamide has also been shown to be impaired (Steinke and Tyler, 1964). These last two studies suggest that the defect lies in the pancreas rather than being due to peripheral loss of muscle mass. Collis and Engel (1968) have, however, reported a similar abnormality of glucose tolerance in other neuromuscular diseases, and they also failed to show an abnormality of the tolbutamide tolerance test in most of their patients. They proposed that the abnormality of glucose metabolism was the result of a decrease in functional muscle mass which leads to the failure of a large glucose load to be stored promptly. This gives rise to raised blood glucose levels. It would appear, however, that this mechanism is unlikely as the liver is the main source for glucose disposal. In addition, it has been shown that, in some forms of motor neurone disease, basal pyruvate concentrations are raised (Cumings, 1962), and that pyruvate concentrations are significantly higher at 60 minutes after a glucose load (Shahani *et al.*, 1971). In the Madras variety of motor neurone disease four patients were found to

have significantly low fasting serum citrate levels, and the ratio of citrate to pyruvate was reduced (Valmiki-rathan *et al.*, 1973).

The purpose of this study was to investigate carbohydrate metabolism in motor neurone disease in more detail, and to investigate whether the abnormal glucose tolerance in that disease is due to an abnormal secretion of insulin, or merely to a decreased tissue mass for glucose storage and metabolism. The patients were also screened for abnormalities of lipid metabolism.

Patients and methods

CLINICAL MATERIAL

Eight patients with motor neurone disease were studied over a four month period. The diagnosis was made on the typical clinical features, and on the results of electromyography or muscle biopsy or both. The clinical features are depicted in Table 1. The control group consisted of eight patients with neurological disease (three Guillain-Barré syndrome, one peripheral neuropathy, two cauda equina lesions, and two cervical paraplegias), who were carefully matched for degree of wasting, physical inactivity, and age. None of the patients were on any drugs at the time of testing, and all had similar dietary backgrounds. None of the patients had symptoms of diabetes or glycosuria on routine testing.

CLINICAL PROCEDURES

All the patients were studied as inpatients and

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Table 1 Clinical features of eight patients with motor neurone disease

Patient	Sex	Age (yr)	Duration (mo)	Pseudobulbar signs	Ambulatory	Wasting	Amylase (Street-Close units)	Albumin (g/l)	Glucose tolerance test interpretation
1	F	46	12	Severe	Yes	++	31	32	Diabetic
2	M	38	24	Severe	No	++	15	29	Diabetic
3	M	52	24	Severe	Yes	Mild	10	17	Non-diabetic
4	M	44	14	Severe	No	++	15	12	Diabetic
5	M	46	12	Severe	No	++	9	17	Borderline
6	M	55	15	Severe	Yes	Mild	9	22	Diabetic
7	F	51	12	Mild	Yes	++	32	13	Non-diabetic
8	M	44	3	None	Yes	Mild	9	25	Diabetic

received a three day preparatory diet containing at least 300 g/day of carbohydrate. Informed consent was obtained from all patients. The following investigations were performed after an overnight fast on consecutive days in the following order:

1. An oral glucose tolerance test was performed using 50 g of glucose, and venous blood samples for glucose and insulin were collected at 0, 60, 120, and 150 minutes after the load.

2. An intravenous tolbutamide tolerance test using 1 g of sodium tolbutamide was performed. Venous blood samples for glucose and insulin were taken at 0, 10, 20, 30, 40, and 60 minutes.

3. Fasting arterial blood was obtained for pyruvate, lactate, and citrate levels. A 50 g glucose load was given, and arterial blood for pyruvate was again taken one hour later. Pyruvate tolerances were performed in six patients with motor neurone disease and six of the controls. Immediately after taking the fasting arterial bloods, and before the administration of the glucose, a lumbar puncture was performed, and cerebrospinal fluid was analysed for glucose, citrate, pyruvate, and lactate.

4. An intravenous insulin tolerance test using 0.1 unit/kg crystalline insulin was performed. Venous blood was taken for glucose estimation at 0, 30, and 60 minutes later.

5. Fasting venous blood samples were taken for estimation of albumin, amylase, cholesterol, and triglyceride determinations.

Laboratory techniques Glucose was measured by autoanalyser. Insulin was measured by the radio-immunoassay method of Morgan and Lazarow (1963) using the Amersham Commercial Kit. Serum citrate was assayed according to the method of Beutler and Yeh (1959). Arterial lactate and pyruvate concentrations were estimated using a semi-micro modification of the Boehringer test kit method (Buchanan and Eyeberg, 1974). Cholesterol was measured by the method of Young and Eastman (1963), and triglycerides by the method of Abell *et al.* (1952). Serum albumin was estimated by the

method of Korngold (1966), and serum amylase according to Street and Close (1956).

Results

The glucose and insulin responses to a 50 g glucose load are given in Table 2.

Statistically there was no difference between the glucose response in the patients with motor neurone disease and the control group (Fig. 1). Only one patient with motor neurone disease (No. 3) had a normal insulin response to the glucose load, and one patient (No. 4) had a delayed response. The other six all had low insulin responses, including one patient (No. 7) with a normal glucose response. Fasting insulin levels in motor neurone disease were significantly depressed compared to controls ($P < 0.05$) as were insulin levels at 60 ($P < 0.01$), and 120 minutes ($P < 0.05$) after glucose loading. Of the eight patients with motor neurone disease, five showed a typical diabetic curve and one had a borderline curve. Of the control group, two had diabetic curves and two had borderline responses. There was no relationship between the degree of glucose intolerance and age of patient, duration of disease, presence of pseudobulbar symptoms, degree of wasting, and whether or not the patient was ambulant.

Table 2 Blood glucose and insulin response to 50 gram glucose loading in patients with motor neurone disease

Patient	Glucose (mmol/l)				Insulin (U/l)			
	F	60'	120'	150'	F	60'	120'	150'
1	5.94	8.58	9.90	6.93	7.2	23.5	25.5	—
2	4.95	6.05	8.25	6.27	3.5	4.2	6.7	11.1
3	5.50	7.48	5.61	4.62	5.0	43.3	13.9	13.4
4	5.06	6.38	6.82	8.14	2.5	14.8	42.0	37.0
5	5.06	7.26	6.60	6.16	2.0	25.0	14.0	7.8
6	6.16	15.40	5.06	4.29	1.9	1.3	1.4	11.9
7	4.84	5.72	5.50	3.74	8.1	29.8	15.5	7.9
8	6.16	13.20	9.02	7.70	4.0	16.5	21.0	17.5
Mean	5.45	8.75	7.09	5.94	3.7	19.8	19.6	14.7
SD	0.55	3.57	1.70	1.60	2.6	13.7	10.4	10.1

Fig. 1 Glucose and insulin response to a 50 gram glucose load in eight patients with motor neurone disease and eight control subjects with neurological disease and an equivalent degree of wasting of muscle.

Fig. 2 Glucose and insulin response to 1 gram sodium tolbutamide in eight patients with motor neurone disease and eight control subjects with neurological disease and an equivalent degree of wasting of muscle.

In response to intravenous tolbutamide there was a significantly greater fall in blood glucose at 10 ($P<0.05$), 20 ($P<0.01$), and 30 minutes ($P<0.05$) in the individuals in the control group compared with those with motor neurone disease (Fig. 2). The maximum percentage fall in blood glucose in response to intravenous tolbutamide was significantly greater ($P<0.05$) in the control group compared with the patients with motor neurone disease (Table 3).

The rise of blood insulin in response to tolbutamide at 10 minutes was significantly greater in the controls ($P<0.01$) compared with the patients with motor neurone disease, as was the maximum insulin level produced in response to tolbutamide ($P<0.01$). The patients with motor neurone disease were more sensitive to exogenous insulin than were the control group ($P<0.05$) (Table 3).

The fasting blood pyruvate levels were less than 113 $\mu\text{mol/l}$ in all the patients with motor neurone disease (Table 4). One of the patients (No. 6) had a

pyruvate value of 176 $\mu\text{mol/l}$ one hour after a glucose load. All the other patients had values less than 113 $\mu\text{mol/l}$. There was no significant difference between the fasting or one hour pyruvate levels in the control or motor neurone disease groups. Similarly, there

Table 3 Results of tolbutamide tolerance test and insulin tolerance test in patients with motor neurone disease

	Tolbutamide tolerance test		Insulin tolerance test
	Max. % fall in blood glucose	Max. insulin level (U/l)	Max. % fall in blood glucose
Motor neurone disease			
Mean	42.0	38.3	68.7
SD	15.0	25.2	8.5
Controls			
Mean	53.7	80.8	55.7
SD	3.9	32.8	16.4
P	<0.05	<0.01	<0.05

Table 4 *Pyruvate, lactate, and citrate values in patients with motor neurone disease*

	Blood					Cerebrospinal fluid				
	Pyruvate (mmol/l)	Lactate (mmol/l)	Lactate/ pyruvate	Citrate (mmol/l)		Glucose (mmol/l)	Pyruvate (mmol/l)	Lactate (mmol/l)	Lactate/ pyruvate	Citrate (mmol/l)
	0	1 hr								
Motor neurone disease										
Mean	0.052	0.066	1.737	33.40	0.180	3.740	0.063	1.859	29.50	0.438
SD	0.020	0.056	0.892	44.60	0.039	4.015	0.027	0.620	22.96	0.207
Controls										
Mean	0.060	0.046	1.845	30.75	0.098	4.070	0.067	1.777	26.52	0.574
SD	0.042	0.024	0.809	19.06	0.055	0.847	0.033	0.367	11.12	0.228
P	NS	NS	NS	NS	< 0.01	NS	NS	NS	NS	NS

was no difference between the blood lactate levels in the two groups. The blood citrate levels were significantly raised in the patients with motor neurone disease when compared with the controls ($P < 0.01$). There was no significant difference in cerebrospinal fluid levels of glucose, lactate, pyruvate, and citrate between the two groups.

Serum triglyceride levels were significantly elevated ($P < 0.05$) in patients with motor neurone disease when compared with the control group (Table 5). There was no difference in serum cholesterol levels. The amylase levels were normal in all patients with motor neurone disease (Table 1). Albumin levels were low but statistically similar in the control group ($19 \text{ g/l} \pm 6$) and in the patients with motor neurone disease ($21 \text{ g/l} \pm 7$).

Table 5 *Cholesterol and triglyceride levels in patients with motor neurone disease*

		Cholesterol (mmol/l)	Triglyceride (mmol/l)
Motor neurone disease	Mean	4.921	1.457
	SD	1.399	0.486
Controls	Mean	4.791	1.050
	SD	0.984	0.283
	P	NS	< 0.05

Discussion

The abnormal glucose tolerance noted in the present study is consistent with the results reported by several investigators in patients with motor neurone disease. When, however, our patients were carefully matched with control patients as far as wasting and lack of physical activity were concerned, no significant difference in glucose tolerance could be demonstrated. This would appear to support the hypothesis of Collis and Engel (1968) that a decrease in functioning muscle mass plays a major role in the pathogenesis of reduced glucose tolerance; a hypothesis further supported by the finding of Gotoh *et al.* (1972) of a

close correlation between the degree of hyperglycaemic response and the extent of muscle wasting.

Patients with motor neurone disease had a low or delayed insulin response to glucose loading while control patients had normal responses or a tendency towards hyperinsulinaemia. In addition, patients with motor neurone disease had a poorer response to tolbutamide, as measured by both insulin and glucose responses, than the control group. Finally, insulin sensitivity tests have indicated that patients with motor neurone disease are more sensitive to insulin than are matched controls.

Gotoh *et al.* (1972), have previously evaluated in detail the reasons why the abnormal insulin response in patients with motor neurone disease cannot be ascribed to pancreatic dysfunction which results from persistent hyperglycaemia. Our data further corroborate their findings in that we had no patients with a combination of abnormal glucose tolerance and normal insulin response and, in addition, one patient with a normal glucose tolerance and an abnormal insulin response. The insulin and glucose responses to tolbutamide further support the hypothesis that the abnormal glucose metabolism in motor neurone disease is primarily due to impaired synthesis or release of insulin, rather than to a disturbed effect of glucose on the β -cell of the pancreas.

In view of the importance of oxidative metabolism as the main source of energy both in the normal activity of nervous tissue and in the maintenance of the myelin sheath, much recent interest has centred around intermediary carbohydrate metabolism in a variety of neurological disorders. Our patients (with one exception) failed to demonstrate the abnormal pyruvate metabolism shown by Cumings (1962) in classical motor neurone disease, and by Valmikirathan *et al.* (1973) in the Madras variety of the disease.

The patients with motor neurone disease demonstrated a significantly raised blood citrate level compared with the control group. This finding is in contrast to that of Valmikirathan *et al.* (1973) who

found low citrate levels in patients with the Madras variety. This is the first study of blood citrate levels in classical motor neurone disease; and these findings would suggest that motor neurone disease-Madras is a distinct entity biochemically as well as clinically (Valmikirathan *et al.*, 1973).

The increased citrate levels in the presence of normal pyruvates is difficult to interpret. All the patients were malnourished as judged by serum albumin levels. It is possible that the raised citrate levels are due to thiamine deficiency with a decreased transketolase activity. The controls, however, were equally malnourished, and this seems to negate this possibility.

There are a number of possible factors in the genesis of the relatively increased triglyceride levels in patients with motor neurone disease. Raised triglyceride levels may be due to increased peripheral clearance associated with insulin deficiency. Decreased physical activity raises blood triglycerides, but this was not a factor in our patients as the controls and patients with motor neurone disease were carefully matched for physical inactivity. Likewise, previous dietary history and use of alcohol were similar in the two groups. A possible explanation for the high triglyceride levels in motor neurone disease compared with the controls is the presence of higher blood citrate levels in the patients with motor neurone disease. Citrate is necessary to transport acetyl Co-enzyme A out of the mitochondria and, in addition, high concentrations of citrate cause the formation of an active form of acetyl CoA carboxylase, an essential enzyme involved in the conversion of acetyl CoA to fatty acids (Bierman and Glomset, 1974).

The results of our studies point to a defect in intermediary metabolism at the citrate level with a possibility of a defect in the Krebs' cycle at this point. Studies of transketolase activity, red cell thiamine, and α -ketoglutarate will be necessary to define the defect further.

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