J. Neurol. Neurosurg. Psychiat., 1967, **30**, 416

Studies of the carrier state in the Duchenne type of muscular dystrophy

Part I Effect of exercise on serum creatine kinase activity

PETER HUDGSON[1], DAVID GARDNER-MEDWIN, R. J. T. PENNINGTON, AND JOHN N. WALTON

From the Departments of Neurology and Neurochemistry, Regional Neurological Centre, Newcastle General Hospital, and the Department of Clinical Chemistry, University of Newcastle upon Tyne

The value of estimating the activities of various serum enzymes, particularly creatine kinase (CK) in confirming the diagnosis of the Duchenne type of progressive muscular dystrophy and in detecting the disease in apparently unaffected younger male sibs of known cases is well established (Chung, Morton, and Peters, 1960; Aebi, Richterich, Stillhart, Colombo, and Rossi, 1961; Pearce, Pennington, and Walton, 1964a). Measurement of serum creatine kinase activity has also been advocated for the diagnosis of the carrier state (Dreyfus, Schapira, and Demos, 1960; Aebi, Richterich, Colombo, and Rossi, 1962; Hughes, 1962; Pearce, Pennington, and Walton, 1964b; Wilson, Evans, and Carter, 1965; Walton and Pennington, 1966) but the rise in serum activity is only slight in the majority of cases and it has recently been shown (Vejjajiva and Teasdale, 1965; Griffiths, 1966) that prolonged strenuous exercise will produce such a rise in normal subjects, though Pearce, Pennington, and Walton (1964c) found no such rise after a short period of moderate exertion. On the other hand, Stephens and Lewin (1965) described three carrier females in whom the serum creatine kinase activity, which was raised during everyday activity, fell to normal after complete bed rest and rose again after exertion. In a further possible carrier the activity in a random serum sample was normal but strenuous exertion again provoked a rise. The purpose of this study was twofold: (1) to determine whether or not a standardized programme of exercise of moderate severity had any effect on serum creatine kinase activity in normal young women; (2) to see if the same standard exercise produced any change in the serum enzyme activity in a group of definite or suspected carriers of Duchenne dystrophy previously shown to have normal serum enzyme levels (Pearce *et al.*, 1964b). It was hoped that a creatine kinase after exercise/creatine kinase after bed rest ratio might prove to be more valuable in carrier detection than random estimation of the activity of this enzyme even if the results were charted on a logarithmic scale on probability paper (Wilson *et al.*, 1965).

MATERIALS AND METHODS

The two groups submitted to the test were drawn from mothers and female sibs of known cases of the Duchenne and late onset X-linked (Becker) types of muscular dystrophy and from young, unmarried female laboratory assistants with no previous history or family history of neuromuscular disease. The former were classified as definite, probable, or possible carriers according to criteria previously laid down by Pearce *et al.* (1964b). One possible carrier who had previously had an elevated serum creatine kinase level was included in the study to assess the effect of exercise on a subject known to have abnormal serum enzyme activity. Each of the carriers and controls was admitted to hospital for a period of two days and two nights and during this time venous blood samples were taken before rising after complete rest in bed for eight hours, after three to four hours of normal activity (this consisted of assisting with domestic chores in the ward, or in the case of controls carrying out their normal laboratory duties), after walking briskly for just over three miles in from 55 to 65 minutes (accompanied by one of us), and after a recovery period of approximately 18 hours (including a further eight hours' sleep). The procedure was duplicated in each case either by prolonging the initial hospital stay or by re-admission after an interval. The serum creatine kinase activity was determined by the method described by Pearce *et al.* (1964c) and was expressed as micromoles of creatine formed from creatine phosphate per hour per millilitre of serum at 37°C. The upper limit of normal in this laboratory has been regarded as 3·5 units (57 international units (I.U./l.) (Pearce *et al.*, 1964c) but we have subsequently obtained figures of slightly more than 4·0 units (66·5 I.U.) very occasionally in normal subjects, though more often in men than in women.

RESULTS

The results of this study are given in Tables I to

[1]Present address: Department of Neurology, Royal Victoria Infirmary, Newcastle upon Tyne.

III. The mean creatine kinase after normal everyday activity is slightly higher than after bed rest in most cases, including both carriers and controls. Very slight rises in serum creatine kinase activity immediately after the standard exercise were recorded in four of the six controls and in all but one of the known and suspected carriers who had normal serum enzyme levels at rest. In no case did the activity rise at this stage above the upper limit of normal. In two of the definite carriers (M.M. and M.W.) the level of activity rose to slightly abnormal levels, 4·0 units (66·5 I.U./1.) and 4·1 units (68 I.U./1.) respectively, 18 hours after the standard exercise. A similar delayed rise was observed in one of the controls (H.C.) who had a serum activity of 20·0 units (332 I.U./1.) 18 hours after exercise. Questioning revealed that she had been swimming for an hour during the evening before the last estimation. The possible significance of this observation is discussed later. Curiously enough she failed to show a similar rise in creatine kinase activity on the day after another period of strenuous swimming; there can be no doubt that the original result was genuine since the assay was repeated. In sharp distinction the possible carrier whose creatine kinase activity was previously known to be elevated at rest showed rises of 2·7 units (43 I.U./1.) immediately after exercise on the first day and 9·1 units (155 I.U./1.) on the second. On both days subsequent falls in her serum creatine kinase activity were recorded after the recovery period.

Taken together, the controls show a significantly higher mean value for serum creatine kinase activity ($P < 0.01$) both immediately and 18 hours after the standard exercise than after bed rest. It is worth noting that the mean elevated values in the controls after the exercise did not return to normal within the 18-hour recovery period. The definite carriers of the gene responsible for the severe Duchenne type muscular dystrophy show a relatively greater mean difference which is not significant. The two definite carriers of the gene of the late onset (Becker) type of X-linked muscular dystrophy showed only slight and again insignificant rises after exertion.

TABLE I

SERUM CREATINE ACTIVITY (UNITS AND I.U./LITRE) IN CONTROLS BEFORE AND AFTER EXERCISE

Subject	*Age in Years*		*Creatine Kinase Activity after Bed Rest*	*Creatine Kinase Activity after Normal Activity*	*Creatine Kinase Activity after Exercise*	*Creatine Kinase Activity after Recovery Period*
M.J.	23	1	2·8 (46·5)[1]	3·0 (50)	3·1 (51·6)	2·3 (38)
		2	1·2 (20)	1·8 (30)	2·0 (33)	1·8 (30)
V.H.	18	1	0·8 (13)	1·6 (27)	1·8 (30)	2·0 (33)
		2	1·3 (21·5)	—	2·3 (38)	2·5 (41·5)
K.H.	18	1	0·8 (13)	1·0 (16·6)	1·9 (31·5)	2·3 (38)
		2	0·8 (13)	1·0 (16·6)	1·5 (25)	1·5 (25)
P.W.	22	1	1·9 (31·5)	2·1 (35)	2·0 (33)	2·3 (38)
		2	0·9 (15)	2·1 (35)	1·9 (31·5)	1·4 (23)
A.B.	21	1	1·2 (20)	1·5 (25)	1·4 (23)	1·7 (28)
		2	0·9 (15)	0·9 (15)	0·9 (15)	0·7 (11·5)
H.C.[2]	19	1	0·7 (11·5)	0·6 (10)	1·1 (18)	0·9 (15)
		2	1·1 (18)	0·3 (5)	1·6 (27)	20·0(332)

1 = first series of observations.
2 = second series of observations.
[1]Figures in brackets are I.U./1.
[2]After swimming for one hour 12 hours before estimation.

TABLE II

SERUM CREATINE KINASE ACTIVITY (UNITS AND I.U./LITRE) IN POSSIBLE CARRIERS BEFORE AND AFTER EXERCISE

Subject	*Age in Years*		*Creatine Kinase Activity after Bed Rest*	*Creatine Kinase Activity after Normal Activity*	*Creatine Kinase Activity after Exercise*	*Creatine Kinase Activity after Recovery Period*
M.B.	20	1	1·6 (27)	1·6 (27)	2·2 (36·5)	1·6 (27)
		2	1·6 (27)	1·9 (31·5)	2·0 (33)	1·8 (30)
J.W.	19	1	1·3 (21·5)	2·7 (45)	2·9 (48)	2·7 (45)
		2	2·7 (45)	2·3 (38)	2·3 (38)	1·9 (31·5)
R.G.	34	1	1·1 (18)	1·6 (27)	2·1 (35)	1·4 (23)
		2	1·4 (23)	1·5 (25)	2·0 (33)	2·8 (46·5)
R.G.	39	1	1·1 (18)	—	1·5 (25)	2·2 (36·5)
		2	2·2 (36)	1·9 (31·5)	2·5 (41·5)	1·8 (30)
D.R.	44	1	1·5 (25)	1·4 (23)	1·7 (28)	1·6 (27)
		2	1·6 (27)	1·7 (28)	1·5 (25)	2·7 (45)
F.E.[1]	41	1	11·7 (194)	12·9 (214)	15·6 (250)	13·3 (216)
		2	13·3 (216)	12·4 (206)	21·5 (357)	18·7 (299)

[1]Case with elevated serum creatine kinase activity at rest.

TABLE III

SERUM CREATINE KINASE ACTIVITY (UNITS AND I.U./LITRE) IN PROBABLE AND DEFINITE CARRIERS BEFORE AND AFTER EXERCISE

Subject	Age in Years		Creatine Kinase Activity after Bed Rest	Creatine Kinase Activity after Normal Activity	Creatine Kinase Activity after Exercise	Creatine Kinase Activity after Recovery Period
Probable Carrier						
J.B.	37	1	1·4 (23)	1·5 (25)	1·4 (23)	1·5 (25)
		2	0·7 (11·5)	0·8 (13)	1·1 (18)	0·9 (15)
Definite Carriers						
R.L.	41	1	1·9 (31·5)	2·2 (36·5)	1·3 (21·5)	2·8 (46·5)
		2	2·8 (46·5)	3·1 (54·5)	2·8 (46·5)	2·7 (45)
M.M.	35	1	1·7 (28)	—	2·1 (35)	4·0 (66·5)
		2	4·0 (66·5)	2·7 (45)	3·4 (56·5)	0·9 (15)
M.W.	27	1	2·3 (38)	2·4 (40)	2·6 (43)	2·2 (36·5)
		2	2·2 (36·5)	1·6 (27)	2·2 (36·5)	4·1 (68)
J.R.[1]	64	1	1·6 (27)	1·7 (28)	2·3 (38)	1·6 (27)
		2	1·6 (27)	2·5 (41·5)	3·0 (50)	2·7 (45)
G.S.[1]	43	1	1·4 (23)	0·9 (15)	1·7 (28)	1·4 (23)
		2	1·4 (23)	1·6 (27)	1·6 (27)	1·4 (23)

[1]Carriers of the late-onset (Becker) type of X-linked muscular dystrophy.

DISCUSSION

There have been a number of recent reports indicating that exercise may produce a rise in the serum level of various enzymes originating from skeletal muscle including serum glutamic-oxaloacetic transaminase (amino-transferase), aldolase, malate dehydrogenase, and lactate dehydrogenase (Henley, Schmidt, and Schmidt, 1960; Halonen and Konttinen, 1962; Poortmans, S'Jongers, Thys, and van Kerchove, 1963). It has also been suggested that the serum creatine kinase activity may similarly vary with exercise in normal subjects (Vejjajiva and Teasdale, 1965; Griffiths, 1966), in patients with muscular dystrophy (Griffiths, 1966), and in female carriers of the gene responsible for the severe sex-linked Duchenne type muscular dystrophy (Stephens and Lewin, 1965). A group of healthy, unmarried, young females carried out a programme of standardized exercise of moderate severity and in no case was the period of exertion followed immediately by a significant rise in serum creatine kinase activity. One of these subjects, however, was found to have a level of 20 units (332 I.U./l.) 18 hours after the exercise, but she had been swimming for one hour during the evening between the two venepunctures. In this context it is noteworthy that in two accounts of variation in serum creatine kinase levels in normal subjects after exercise (Vejjajiva and Teasdale, 1965; Griffiths, 1966) the rises were produced by violent or greatly prolonged exertion. This may well account for the abnormal activity found in the serum in our single control subject after swimming. Such violent exercise is unusual in the daily life of a suspected carrier and provided it is stressed that activity of this type should be avoided at or about the time when a blood sample is due to be taken, it is therefore irrelevant to the problem of carrier detection. The rise in creatine kinase recorded after exercise in the possible carrier with the high resting level is in sharp contrast to the findings in the group with the normal resting levels. This rise probably reflects quantitative differences in the permeability characteristics of the sarcolemmal membranes.

With the methods available at present a success rate of approximately 70% can be expected in detection of the carrier state in Duchenne type muscular dystrophy using serum creatine kinase estimation alone. In the hope of finding a valid and practical means of provoking a rise in serum creatine kinase activity in such carrier females a number of known and suspected carriers with normal basal serum creatine kinase levels have been subjected to the same test as the normal controls previously described. Slight but insignificant rises occurred after exercise in all the subjects tested but in only two cases (both known to be definite carriers on genetic evidence) did the level rise to just above the upper limit of normal for this laboratory. However, in neither of these women was the rise in activity significantly greater than that seen in normal control subjects. We have concluded therefore that serum creatine kinase activity must be elevated under basal conditions before a diagnosis of the carrier state can be made with reasonable confidence. Furthermore our results suggest that moderate exercise of the type described is unlikely to be a consistently reliable provocative measure in putative carriers with normal resting serum creatine kinase activity.

Although our results suggest that in females carrying the gene responsible for the severe Duchenne type of muscular dystrophy the rise in serum creatine kinase activity is sometimes slightly greater than in control subjects, the difference is not

statistically significant. And our findings do not confirm the tendency shown in the three cases of Stephens and Lewin (1965) in which a greater than normal rise during everyday activity was demonstrated. We would therefore conclude that in suspected female carriers who show a normal resting serum creatine kinase level, moderate exercise is inadequate as a provocative test in increasing the carrier detection rate.

It must also be emphasized that since in normal control individuals we have demonstrated a slight but significant rise in serum creatine kinase activity 18 hours after moderate exercise and in one case a very substantial rise after vigorous and prolonged exertion, suspected carriers should be warned against indulging in any form of unusual or excessive physical activity on the day before blood samples for the estimation of serum creatine kinase are to be taken.

SUMMARY

Estimations of the serum creatine kinase activity have been made in 10 females relatives (mothers or sibs) of known cases of the severe Duchenne type of muscular dystrophy, in two definite carriers of the gene responsible for the late onset (Becker) type of X-linked dystrophy, and in six normal controls. The female relatives were classified as definite, probable, or possible carriers on genetic grounds and in only one of them was the serum creatine kinase activity known to be increased at rest. These individuals were chosen specifically in the hope that a provocative exercise test would be shown to increase serum creatine kinase activity to a greater degree in carriers than in controls. The observations were made after rest, during normal activity, and after a standard exercise (walking briskly for three miles). Slight rises in serum creatine kinase levels after exercise were found in the majority either immediately after exercise or sometimes to an even greater extent 18 hours later; however, no definite trend could be discerned in either group. In one control patient a rise of 19 units (315 I.U./1.) was probably produced by swimming 12 hours earlier. It has been concluded that moderate exercise does not provoke a consistent rise in serum creatine kinase levels in either normal subjects or in carriers of the gene responsible for either form of X-linked muscular dystrophy and that severe exercise should be avoided for 36 hours before blood samples are taken for carrier detection purposes.

We wish to express our gratitude to Miss C. Taylor for technical help and to Miss R. Allan and Miss P. Ruddick for secretarial assistance. This work was carried out with the assistance of grants from the Medical Research Council, the Muscular Dystrophy Group of Great Britain, and the Muscular Dystrophy Associations of America, Inc.

REFERENCES

Aebi, U., Richterich, R., Colombo, J. P., and Rossi, E. (1962). Progressive muscular dystrophy: II. Biochemical identification of the carrier state in the recessive sex-linked juvenile (Duchenne) type by serum creatine-phosphokinase determinations. *Enzym. biol. clin.*, **1**, 61-74.

——, ——, Stillhart, H., Colombo, J. P., and Rossi, E. (1961). Progressive Muskeldystrophie: III. Serumenzyme bei der Muskeldystrophie im Kindesalter. *Helvet. paediat. Acta*, **16**, 543-564.

Chung, C. S., Morton, N. E., and Peters, H. A. (1960). Serum enzymes and genetic carriers in muscular dystrophy. *Amer. J. hum. Genet.*, **12**, 52-66.

Dreyfus, J.-C., Schapira, G., and Demos, J. (1960). Étude de la créatine-kinase sérique chez les myopathes et leurs familles. *Rev. franç. Étud. clin. biol.*, **5**, 384-386.

Griffiths, P. D. (1966). Serum levels of ATP: creatine phosphotransferase (creatine kinase). The normal range and effect of muscular activity. *Clin. chim. Acta*, **13**, 413-420.

Halonen, P. I., and Konttinen, A. (1962). Effect of physical exercise on some enzymes in the serum. *Nature (Lond.)*, **193**, 942-944.

Henley, K. S., Schmidt, E., and Schmidt, F. W. (1960). Serum enzymes. *J. Amer. med. Ass.*, **174**, 977-981.

Hughes, B. P. (1962). Serum enzymes in carriers of muscular dystrophy. *Brit. med. J.*, **2**, 963-964.

Pearce, J. M. S., Pennington, R. J. T., and Walton, J. N. (1964a). Serum enzyme studies in muscle disease: II. Serum creatine kinase in muscular dystrophy and in other myopathic and neuropathic disorders. *J. Neurol. Neurosurg. Psychiat.*, **27**, 96-99.

——, ——, —— (1964b). Serum enzyme studies in muscle disease: III. Serum creatine kinase activity in relatives of patients with the Duchenne type of muscular dystrophy. *Ibid.*, **27**, 181-185.

——, ——, —— (1964c). Serum enzyme studies in muscle disease: I. Variations in serum creatine kinase activity in normal individuals. *Ibid.*, **27**, 1-4.

Poortmans, J., S'Jongers, J. J., Thys, A., and van Kerchove, E. (1963). L'activité transaminasique dans le sang total et dans le sérum au cours de l'effort musculaire. *Rev. franç. Étud. clin. biol.*, **8**, 173-175.

Stephens, J., and Lewin, E. (1965). Serum enzyme variations and histological abnormalities in the carrier state in Duchenne dystrophy. *J. Neurol. Neurosurg. Psychiat.*, **28**, 104-108.

Vejjajiva, A., and Teasdale, G. M. (1965). Serum creatine kinase and physical exercise. *Brit. med. J.*, **1**, 1653-1654.

Walton, J. N., and Pennington, R. J. T. (1966). Studies on human muscular dystrophy with particular reference to methods of carrier detection. *Ann. N.Y. Acad. Sci.*, **138**, 315-328.

Wilson, K. M., Evans, K. A., and Carter, C. O. (1965). Creatine kinase levels in women who carry genes for three types of muscular dystrophy. *Brit. med. J.*, **1**, 750-753.