Effect of exercise on serum creatine kinase in carriers of Duchenne muscular dystrophy

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SUMMARY In order to evaluate the effect of exercise on serum creatine kinase levels, blood samples were obtained from 17 normal females and 12 Duchenne muscular dystrophy carriers before and 9 hours after moderately strenuous exercise. The results revealed that after exercise serum creatine kinase levels may be better indicators of carrier status than resting levels. The mean serum creatine kinase levels before and after exercise, as well as the mean increases, were found to be significantly greater in Duchenne muscular dystrophy carriers than in normal control subjects.

Numerous studies have been concerned with the identification of carriers of Duchenne muscular dystrophy. Past investigators have focused on the rise in serum enzymes,¹⁻³ abnormal muscle ultrastructure,⁴ myopathic changes of the electromyogram,⁵ derangement in muscle protein synthesis,⁶ and muscle weakness.⁷ Other studies reported aberrations in the electrocardiogram,⁸ red blood cell morphology,⁹ membrane protein peak II phosphorylation activity,^{10 11} and lymphocyte capping,¹² in carriers of this X linked neuromuscular disorder. Unfortunately, many of these pathological findings are not observed in all carriers of Duchenne muscular dystrophy, some of the tests are cumbersome or invasive in nature, and other studies which have not been duplicated await confirmation.

At present, serum creatine kinase determination is the most widely used method for carrier detection^{13 14} though it has been proposed that pyruvate kinase may be a more sensitive indicator.² Since there is considerable variation in serum creatine kinase levels among known carriers, several enzyme determinations are usually made in the attempt to establish carrier status.¹⁵ During the days before testing, undue motor activity is usually avoided¹³ because it has been shown that moderately heavy exercise will result in increased serum creatine kinase levels.¹⁶⁻¹⁹

This study was directed towards the evaluation of the hypothesis that the serum creatine kinase concentration several hours after a measured exercise would constitute a more effective carrier detection method.

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Subjects and methods

Seventeen healthy female volunteers, between the ages of 20 and 41, who did not have clinical muscle weakness, participated in this study as controls. Obligate, probable, and possible carriers known to us through contacts with Duchenne muscular dystrophy patients followed at the Child Development Center were invited to take part in this investigation. Both control and study populations were asked to refrain from unusual exercise for 2 days before testing. The study design, rationale, and test procedure were explained and the subjects gave written informed consent.

On the morning of the test, the participants' weight was recorded, a venous blood sample was drawn, and the subjects were asked to pedal a Schwinn EX-1 Bicycle Ergometer to produce 5000 to 6000 kilopond-metres of work within a 10 to 12 minute period. The exercise load setting, which was 900 kilopond-metres/minute at the beginning was reduced to 600 and later to 450 kilopond-metres/minute when the subjects became exhausted at the initial higher exercise levels. After completion of the exercise, the participants were permitted to go about their usual daily activities with restriction only of undue exercise. Based on the evaluation of previous reports,¹⁶⁻²⁰ we obtained a second venous blood sample, approximately 9 hours after completion of the exercise.

All blood specimens were centrifuged and the serum was frozen. Serum creatine kinase determinations were carried out within 48 hours at 37°C, according to the method of Szasz *et al.*^{21 22}

Results

Table 1 presents the age, weight, total exercise load, and serum creatine kinase activity immediately before and 9 hours after exercise of 17 normal appearing control females. Equivalent data for female relatives of patients with Duchenne muscular dystrophy are shown in table 2. In genetic terms, these include definite, probable, and possible carriers. However, based on pedigree evaluations and previous significantly increased serum creatine kinase levels, it is assumed that the subjects listed in table 2 are Duchenne muscular dystrophy carriers. Table 3 contrasts the mean serum creatine kinase levels before and 9 hours after exercise as well as the mean increases of serum creatine kinase between the controls and carriers. These data indicate that the after exercise values may be more discriminating than both the before exercise levels and the net change between the first and second blood samples.

A graphic presentation of a discriminant function

TABLE 3 Comparison between serum creatine kinase (CK) levels of controls and carriers

	CK (IU) before exercise (mean $\pm$ 1SD)	CK (IU) after exercise (mean $\pm$ 1SD)	Delta CK (IU) (mean $\pm$ 1SD)
Controls	56 $\pm$ 19	68 $\pm$ 26	12 $\pm$ 19
Carriers	373 $\pm$ 394	532 $\pm$ 505	159 $\pm$ 161
p value	<0.025	<0.015	<0.02

analysis is depicted in the figure. A log log transformation was used because of the extreme skewness of the distributions. The X near the centre of the graph indicates a mid-point between the means of the two groups. The classification boundary is shown by the broken line, which is the boundary that would be expected to produce the minimum misclassification. Since the calculated boundary is more horizontal than vertical, it implies that the after exercise value is more important than the before exercise value in making the discrimination.

TABLE 1 Age, weight, total exercise load, and serum creatine kinase (CK) determinations in normal appearing control subjects

Subject	Age (yr)	Weight (kg)	Exercise (kpm)	CK (IU) before exercise	CK (IU) after exercise	CK (IU) change
1	37	56.4	5775	42	42	0
2	25	49	6750	73	79	+6
3	27	53	6000	58	47	-9
4	22	50	6750	39	42	+13
5	26	49	5350	40	103	+63
6	26	49.5	6675	55	44	-9
7	34	56.4	6150	35	46	+11
8	38	76.5	6100	78	92	+14
9	26	63	6490	44	49	+5
10	32	56	6750	68	80	+12
11	36	63.3	5624	55	108	+53
12	31	53.3	6750	52	65	+13
13	30	70	4565	77	73	-4
14	34	72.6	7200	62	73	+11
15	41	62.8	5300	25	20	-5
16	20	47	6700	105	113	+8
17	32	58	5500	49	73	+24

TABLE 2 Age, weight, total exercise load, and serum creatine kinase (CK) determinations in female relatives of patients with Duchenne muscular dystrophy

Subject*	Affected relatives	Age (yr)	Weight (kg)	Exercise (kpm)	CK (IU) before exercise	CK (IU) after exercise	Increase of CK (IU)
1	2 sons	34	52	5550	175	365	+190
2	2 sons	50	51	3787	164	188	+24
3	3 sons	42	65	6975	52	88	+36
4	3 brothers, 1 son	32	55	5700	178	320	+142
5	2 sons	43	67	5475	106	148	+42
6	1 uncle, 1 son	45	80	5075	160	207	+47
7	2 brothers	17	55	5525	1515	1994	+479
8	2 brothers	23	58	5650	181	220	+39
9	3 uncles, 1 brother	13	51	6250	652	729	+77
10	1 brother	18	55	5550	229	530	+301
11	1 brother	19	54	5575	668	729	+61
12	1 brother	16	78	6900	400	871	+471

*According to pedigree evaluation subjects 7 to 12 are possible carriers of Duchenne muscular dystrophy. Because of their significantly raised serum kinase levels they are considered to be definite carriers in this study.

FIGURE Serum creatine kinase levels (log log transformation) in normal females (○) and in carriers of Duchenne muscular dystrophy (●) before and after exercise. - - - Discriminant boundary.

Discussion

In this study we investigated the possibility of improving the discriminating ability of serum creatine kinase by taking blood samples before and 9 hours after a measured exercise load. As previous reports¹⁶⁻²⁰ indicate, the amount of exercise is an important consideration. Emery²³ reported preliminary results similar to ours, stressing the need for further study of the effects of standard exercise. Thomson²⁰ measured creatine kinase increases after a more leisurely walk than Emery's subjects. He was not able to demonstrate a significant exercise effect. Hughes *et al*²⁴ found no significant difference in bicycle exercise effects in carriers compared with controls. However, these subjects exercised somewhat less than ours, and the creatine kinase determinations were not made between 4 and 20 hours after exercise when the maximum exercise effects would be expected. In the study of Nuttal and Jones,¹⁹ normal untrained female subjects displayed increased creatine kinase levels 8 to 16 hours after strenuous weight lifting. The exercise load given to the subjects in our study was probably between the latter two in difficulty. Although two of our control subjects had large increases in their creatine kinase after exercise, the results did not exceed the normal range (15 to 135 IU) in our laboratory. The exercise load of our subjects

seems to be sufficient to improve the discriminating ability of creatine kinase levels. While these preliminary data provide evidence that after exercise serum creatine kinase determinations are of greater value in identifying carriers of Duchenne muscular dystrophy than randomly obtained levels, we propose a large scale study to investigate further and substantiate our result.

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