

Serum creatine kinase levels in pubertal, mature, pregnant, and postmenopausal women

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SUMMARY In order to obtain control values for the diagnosis of carriers of Duchenne muscular dystrophy, creatine kinase (CK) estimations were performed in two laboratories on 148 healthy teenage girls (of whom 38 were premenarchal and 110 postmenarchal), 133 healthy mature women, 124 pregnant women, and 37 postmenopausal women. These levels were highest in the premenarchal teenagers, and became successively lower in the postmenarchal teenagers, the mature women, and the pregnant women, so that the mean level of the pregnant women was less than half that of the teenagers. The CK levels then rose again after the menopause. If the distribution of CK levels in adult non-pregnant women had been taken as controls for teenagers who were possible carriers for Duchenne muscular dystrophy, then one third of those teenagers classified as carriers would have been so classified incorrectly.

Levels of serum creatine kinase (CK) are used in the distinction of carriers of X-linked muscular dystrophies. Therefore, as part of a genetic study of Duchenne muscular dystrophy in the West Midlands, CK levels in healthy females were studied. Four groups of controls were investigated: teenage girls (subsequently divided into those who were pre- or postmenarchal); healthy non-pregnant women aged between 20 and 40 years; women who were in the first 16 weeks of pregnancy; and postmenopausal women. We chose these groups in view of the need to define control distributions, including youth, pregnancy, and those ages representative of grandmothers whose status are often of value in defining the time of mutation.

The blood was studied on the understanding with the clinicians involved, the girls who gave it, and their parents who allowed it, that under no circumstances would any results be made available. All our records of identity have now been destroyed. It is very unlikely that any carrier would be found in a few hundred women, since the incidence of carriers cannot exceed twice the incidence of disease in males, which is about 1 in 4000 in the West Midlands.

Material and methods

The teenage controls were 148 girl volunteers from a

nearby day school. They were bled during the lunch hour having refrained from games on the morning of the test. They had also been asked not to give blood if they had had a minor illness, accident, or unusual exertion in the previous two days. Each girl was asked whether her periods had started and, if so, whether she was menstruating that day.

The 133 healthy non-pregnant females consisted of 124 nurses at the Birmingham Maternity Hospital, who were being tested for rubella antibodies, and 9 graduate and secretarial volunteers from the Infant Development Unit, who were bled during a normal working day. The combined group of adult women aged 20 to 40 years will henceforward be termed 'nurses'. Blood was made available from that taken on booking from 124 pregnant women whose gestation was 16 weeks or less. The blood from women aged over 55 was acquired through the assistance of the Blood Transfusion Centre. About 5 ml blood from donors who were born in 1923 or earlier were put into separate tubes and the serum taken off when the session ended.

The sera from all the controls were thus separated on the day of taking. They were split into two so that CK estimations could be carried out in two laboratories: the Midland Centre for Neurosurgery and Neurology (MCNN) and the Queen Elizabeth Medical Centre (QEMC). Estimations on sera from the nurses and pregnant women were performed on

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the same specimens within two days; until then the sera was stored at 4°C. These women were not known to be controls by the biochemists who tested the samples. Sera from the teenage girls and from the elderly blood donors were frozen once, and the estimations were performed in a group.

The method of CK estimation used at the MCNN was the continuous flow version of Hughes's method (Hughes, 1962; Siegel and Cohen, 1966), which is based on the colorimetric determination of the creatine liberated from creatine phosphate at 37°C. This method has been in use for nearly 20 years in muscular dystrophy laboratories, and was selected because its greatest precision is at the levels intermediate between those usual for controls and those for carriers of X-linked muscular dystrophy, and because it is suitable for large batches. The method used at the QEMC was the Boehringer CK 'activated' U-V method for automated analysis, employing the Vitatron AKES at 30°C. This method measures spectrophotometrically the production of NADPH, which is ultimately a measure of the amount of ATP formed when CK splits creatine phosphate in the presence of ADP (Table 1). This method is more specific than Hughes's method, but it is essential that serum freshly separated from red cells is used, since ATP and glucose-6-phosphate

present in the red cells may leak out and produce falsely high results. The method is faster than the continuous flow procedure outlined above and is most precise for very high values.

Since the distribution of CK levels is not symmetrical about the mean, we have, like most other workers, used the logarithms of values for our graphical display and the calculation of the means, which are therefore geometrical means. The 'two standard deviations above the mean' is based on the logarithms of the values, on the assumption that they are normal. In fact, there were occasional erratic high values which are outside the likely Gaussian approximation. These were not excluded from the calculations. Attempts to improve normality by other transformations produced limited improvements in fit at a disproportionate cost in complexity.

Results

The schoolgirls were aged 12 to 16, the nurses 20 to 40 (with a mean of 24 years), the pregnant women 17 to 41, and the blood donors, who were assumed to be postmenopausal, were aged 55 years or over. Within the separate groups, there was no evidence of regression of CK level with age except in the schoolgirls, where a small negative regression could be fully accounted for by the menarche.

The results of CK estimations are given in Table 2 and presented graphically in Fig. 1 and 2. The highest levels were found in the premenarchal schoolgirls, then the postmenarchal schoolgirls, then the nurses, and then the pregnant women, whose levels were so much lower that their upper limit of 2 SD was below the mean for the schoolgirls. The levels in the postmenopausal women increased to about that of the postmenarchal girls.

Of the schoolgirls, 38 (aged 12 to 16, with a mean age of 13.3 years) had not yet begun to menstruate.

Table 1 Summary of CK metabolism to illustrate estimation procedures

CP + ADP	CK	C + ATP
	HK	
ATP + G	G6PD	G-6-P + ADP
G-6-P + NADP ⁺		Glu-6-P + NADPH + H ⁺

CP, creatine phosphate; ADP, adenosine diphosphate; CK, creatine kinase; C, creatine; ATP, adenosine triphosphate; G, glucose; HK, hexokinase; G-6-P, glucose-6-phosphate; NADP⁺, nicotinamide adenine dinucleotide phosphate; G6PD, glucose-6-phosphate dehydrogenase; Glu-6-P, gluconate-6-phosphate; NADPH, reduced NADP⁺.

Table 2 CK levels in 5 groups of females

	Teenage girls Premenarchal	Postmenarchal	Nurses (20-40 y)	Pregnant women	Women aged over 55
<i>Results from MCNN</i>					
No	38	110	133	124	37
Mean of data (logs)	1.740	1.634	1.520	1.396	1.644
SD of data (logs)	0.156	0.170	0.175	0.152	0.129
Antilog of mean (IU/l)	55.5	43.0	33.1	24.9	44.0
2 SD range (IU/l)	26.8-101.2	19.7-94.2	14.8-74.1	12.4-50.1	24.3-79.6
Ratio of (mean + 2 SD) to mean	1.8	2.2	2.2	2.0	1.8
No of results over 75 IU/l	6 (16%)	9 (8%)	1 (1%)	1 (1%)	1 (3%)
<i>Results from QEMC</i>					
No	38	109	126	113	37
Mean of data (logs)	1.672	1.554	1.377	1.215	1.508
SD of data (logs)	0.187	0.196	0.252	0.201	0.187
Antilog of mean (IU/l)	47.0	35.8	23.8	16.4	32.2
2 SD range (IU/l)	19.9-111.2	14.5-88.3	7.5-76.0	6.5-41.4	13.6-76.2
Ratio of (mean + 2 SD) to mean	2.4	2.5	3.2	2.5	2.4
No of results over 75 IU/l	5 (13%)	6 (6%)	3 (3%)	1 (1%)	1 (3%)

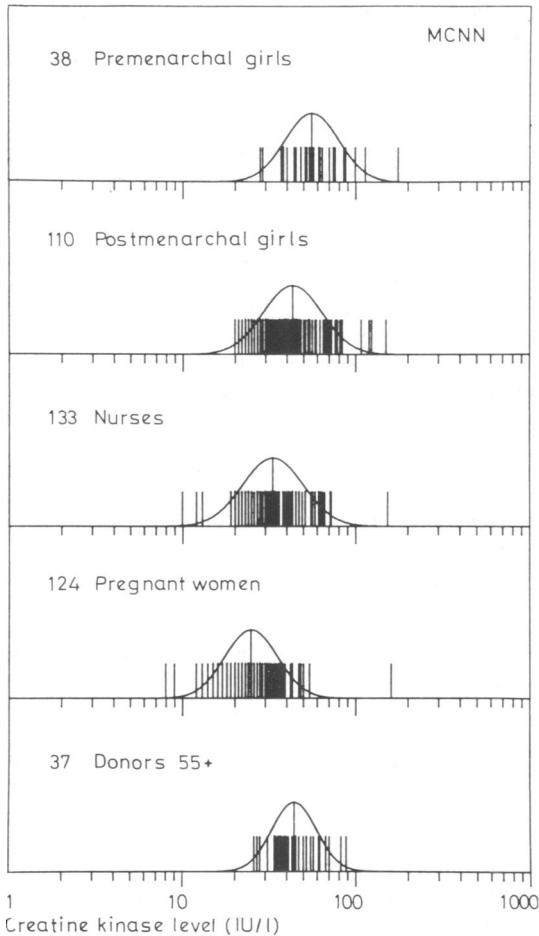


Fig. 1 Distributions of the MCNN CK levels of (from top downwards) premenarchal girls, postmenarchal girls, mature women, pregnant women, and postmenopausal women.

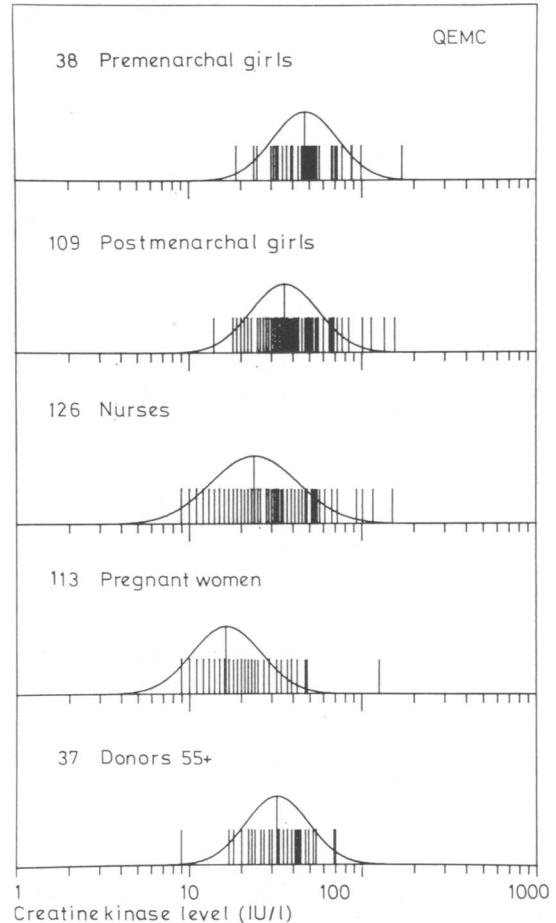


Fig. 2 Distributions of the QEMC CK levels of (from top downwards) premenarchal girls, postmenarchal girls, mature women, pregnant women, and postmenopausal women.

Their mean CK levels were more than $1\frac{1}{2}$ times the means of the nurses, and more than twice the means of the pregnant women (Table 2). The 110 school-girls who had started to menstruate had CK levels that were intermediate between those of the premenarchal girls and the nurses. The 21 girls who were menstruating on the day their blood was taken had CK levels that were distributed fairly randomly among the postmenarchal group, with a mean of 46.6 IU/l compared to 39.5 IU/l in the others.

The 124 pregnant women, whose gestations ranged from 6 to 16 weeks, had mean CK levels of 24.9 and 16.4 IU/l. There was no regression of CK level on gestation within their dates. In two women we studied CK levels before and after conception. In

one, the CK level had fallen from previous levels of 36, 35, and 36 IU/l to 24 IU/l on the first day of her first missed period, and continued to be low throughout her pregnancy (14, 22, 16, 23, 23 IU/l). In the other woman, there was a smaller fall from pre-pregnancy levels of 34, 24, 23, and 23 IU/l to pregnancy levels of 20 and 19 IU/l before she had a spontaneous abortion at 10 weeks.

Discussion

The diagnosis of potential carriers of Duchenne muscular dystrophy cannot yet be made with certainty, and the level of creatine kinase in the serum is the most informative test on the likelihood

of an individual being a carrier. The inference, after allowance for the likelihood based on relationship, is based on a comparison with normal levels, which are often assumed to be largely uninfluenced by age. However, we have found far higher levels of CK in healthy young girls than in mature women, substantially lower levels of CK in pregnant women (also shown by King *et al.*, 1972), and higher values again after the menopause (also observed by Sweetin and Thomson, 1973).

Several groups of workers (Thompson *et al.*, 1967; Perry and Fraser, 1973; Moser and Vogt, 1974; Zatz *et al.*, 1976) have suggested that teenage carriers of Duchenne muscular dystrophy have higher levels of CK than adult carriers. However, the evidence for this has been indirect, since a carrier can rarely, if ever, be recognised when in her teens as she would have had to have had an affected son. Thompson *et al.* (1967) and Zatz *et al.* (1976) observed that while about two-thirds of known carriers for Duchenne muscular dystrophy had raised CK levels, the number of sisters of patients who had raised CK levels was similar to the number expected to be carriers on the basis of their mother's status. Perry and Fraser (1973) and Zatz *et al.* (1976) have reported families in which a mother has had normal CK levels and a daughter high levels. Indeed, Zatz *et al.* (1975) have suggested that such mothers should be considered to be carriers on the basis of their daughter's results. Moser and Vogt (1974) followed CK levels in 19 definite and possible carriers for Duchenne muscular dystrophy over a 13-year period. They found that during this time the number of females with high CK levels had fallen from 14 in 1961 (5 definite and 9 possible carriers) to 6 in 1974 (3 definite and 3 possible carriers). These changes could not be attributed to variation in laboratory technique, but were explained by changes of CK levels with age. Subsequently, Moser (1977, 1978, personal communication) examined carrier detection rates using CK levels in 116 female relatives, and compared the results to the numbers of carriers expected from detailed pedigree analysis. He found that while carrier detection was 65% of that expected after the age of 20 (in accordance with other workers), it was 91% under the age of 20. He therefore advocates estimating CK levels on female relatives when they are young. However, such analysis gives no information on whether those classified as carriers are correctly classified, but only that any false-positives are balanced by similar numbers of false-negatives.

If young girls are going to be tested and advice given, it is essential that their CK levels are compared with those of a similar population. The higher CK levels reported here among teenage girls raises the

possibility that the higher detection rate in young girls may partly result from the inclusion of false-positives. It cannot be known whether the CK levels of teenage carriers for Duchenne muscular dystrophy are raised to the same extent as those of normal teenagers, or whether to a much greater extent. However, it seems reasonable to assume that they will be raised and that the level of mean plus 2SD is a satisfactory point at which to divide possible carriers into probable carriers or unlikely carriers. In a study of Duchenne muscular dystrophy (Bunday *et al.*, 1978), there were 52 possible carriers under the age of 20. On the basis of individual pedigree analysis, together with assessment of the CK values in their mothers, 17 to 18 of these girls were likely to be carriers. Of these, 16 had CK levels which were above the 2SD range for their appropriate controls. If the levels in those 52 girls had been compared to those of an adult female population, 23 would have been classified as carriers, 7 of them presumably incorrectly.

The lowering of CK levels in early pregnancy may be analogous to the effect of the menarche, but is difficult to explain. Very few proteins are reduced in pregnancy. The lowering of CK levels in pregnancy in carriers was observed by Blyth and Hughes (1971) and Emery and King (1971), and King *et al.* (1972) subsequently described a lowering of CK levels in pregnant normal women. The present study not only shows a substantial reduction of CK levels in early pregnancy, but, on the findings in one woman, suggests that this reduction may have occurred by the time pregnancy is suspected. It will be difficult to obtain adequate numbers of pregnant obligate carriers to define their range of CK levels at all accurately.

These effects of puberty, pregnancy, and the menopause on CK levels in healthy women raise the possibility that other hormonal influences, such as menstruation and the contraceptive pill, may alter CK levels. However, previous workers have noted no effect of menstruation (Pearce *et al.*, 1964; Wilson *et al.*, 1965; Perry and Fraser, 1973), though Sweetin and Thomson (1973) observed higher CK levels in the second half of the menstrual cycle. Similarly, previous workers have found no effect of the contraceptive pill on CK levels (Perry and Fraser, 1973; Simpson *et al.*, 1974). In the present study, an appreciable proportion of the nurses was likely to be using oral contraceptives, but we have no information on this.

The numbers of results in each group which exceeded two SD (Table 2) were greater than the proportion defined by a Gaussian distribution. In no group would it be possible to infer the upper centile distribution from the Gaussian approximation.

However, there might have been fewer outlying results had it been possible to make several estimations on the same individual, as is recommended when testing possible carriers (Wilson *et al.*, 1965).

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