Emery–Dreifuss muscular dystrophy (EDMD) is a rare X-linked muscular dystrophy. Creatine kinase (CK) activity is increased in serum of affected males, but results for aldolase and lactate dehydrogenase (LD) in serum have been inconsistent, as have those for CK in carrier females. There have been few studies of CK-MB or LD isoenzyme-1 (LD-1) in EDMD. We measured CK, CK-MB, LD, LD-1, and aldolase activity in sera of 64 members of two large families with EDMD. DNA analysis had been carried out on all subjects. Although CK, LD, and aldolase activities were significantly increased in affected males, CK activity was the most consistently increased and was the least subject to artifactual increases. Mean CK-MB in serum was mildly increased, but LD-1 was within the normal reference interval, suggesting that CK-MB is increased in skeletal muscle in EDMD, as has been found in other forms of dystrophy. CK decreased with age in affected males. We saw no significant increases of muscle enzymes or isoenzymes in 33 EDMD carriers studied, of whom 19 were obligate carriers and 14 had been identified by DNA analysis.

Additional Keyphrases: creatine kinase; aldolase; lactate dehydrogenase; heritable disorders; DNA probes; age-related effects

Emery–Dreifuss muscular dystrophy (EDMD) is a rare X-linked muscular dystrophy characterized by slowly progressive muscle weakness, early joint contractures, and atrial arrhythmias. The disorder was first reported in 1961 by Dreifuss and Hogan (1). The cardiac findings and results for serum creatine kinase (CK; EC 2.7.3.2) in the same family were reported in 1966 by Emery and Dreifuss (2). Creatine kinase is increased in EDMD, but not to the extremely high values frequently seen in Duchenne muscular dystrophy (DMD). Activity of lactate dehydrogenase (LD; EC 1.1.1.27) in serum in EDMD reportedly is within normal limits or slightly increased (3–10). Aldolase (EC 4.1.2.13; fructose-bisphosphate aldolase) activity also has been reported to be normal (9) to slightly increased (7). Despite reports of cardiomyopathy in this disorder (3, 11), the few reported values for CK-MB have been within normal limits (5, 12). Studies of serum LD-1, the major LD isoenzyme in cardiac muscle, have not been reported in EDMD. Although some authors (6, 13–15) have reported normal CK activities in obligate carrier females of EDMD, others (2, 16–19) have reported mild increases in as many as 36% of such subjects. These studies involved compara-}

Materials and Methods

We studied the family reported in 1966 by Emery and Dreifuss (2), hereafter referred to as Family 1, and the family reported in 1979 by Hassan et al. (3), hereafter referred to as Family 2. After updating the pedigrees, we obtained blood for assays of CK, CK-MB, LD, LD-1, and aldolase from affected males, possibly affected males, obligate carrier females, possible carrier females, and other members of the families, a total of 84 samples. DNA analysis was performed concurrently on samples from all subjects whose enzymes were measured, and the results were used to determine the status of possibly affected males and possible carrier females (20). Complete results of the DNA studies will be reported in detail elsewhere. Serum was obtained and stored for up to two days at either 4 °C (LD, LD-1) or –20 °C (CK, CK-MB, aldolase) until assayed. To measure CK, we used the optimized reverse kinetic assay (21) with EDTA, to obviate interference from calcium (22), and a two-part reagent (23). CK-MB was quantified by the immunoinhibition-immunoprecipitation method (24) as described by Bruns et al. (25) and modified for use at 37 °C (26). LD and LD-1 were measured as described by Bruns et al. (27, 28), but at 37 °C. Aldolase was measured by a conventional kinetic ultraviolet absorbance method. All enzyme and isoenzyme measurements were performed under strict temperature control (±0.1 °C), by kinetic methods with computer-calculated rates of reaction. Statistical significance was tested by the nonparametric Wilcoxon–Mann–Whitney U test, comparing affected males with normal males, and carrier females with normal females, all of whom were family members and from whom blood samples were drawn at the same times. Because the normal reference ranges for CK and LD activity in serum vary with age, we also calculated the values as multiples of the upper limit of normal for age to facilitate statistical comparison of the groups. To facilitate comparison with present values, we also calculated the values of CK reported by Emery and Dreifuss (2) and McKusick (9), determined by different methods, as multiples of the upper limit of normal.
Results

Of the 16 obligate carrier females identified by Emery and Dreifuss (2), we were able to obtain samples from 13 (two were dead and one was unavailable). We also identified and obtained samples from three additional obligate carriers and from six probable carriers in Family 1, the latter having been identified by DNA analysis. Of the 11 obligate carriers identified by Hassan et al. (3), we obtained samples from two (five were dead, three were unavailable, and one was not truly a carrier). We also identified two additional obligate carriers in Family 2 (obtaining a serum sample for muscle enzyme analysis from one of them) and used DNA analysis to identify nine probable carriers (obtaining a serum sample for muscle enzyme analysis from eight).

Table 1 shows CK, CK-MB, LD, LD-1, and aldolase values for seven affected males (three newly identified) in Family 1 and for three affected males in Family 2. It also shows the mean values for affected males, obligate carrier females, obligate + probable (diagnosed by DNA analysis) carrier females, and normal males and females. CK, LD, and aldolase activities were all significantly greater in affected males than in unaffected males. However, an increase in LD was seen only when the values were adjusted for age by use of multiples of the upper limit of normal. Among these enzymes, CK was most frequently increased above the reference interval: in nine of 10 compared with three of 10 for LD and aldolase, respectively.

LD and aldolase appeared less suited to assay in field clinics. Several samples that could not be centrifuged until approximately 12 h after they were obtained were kept on ice. About half of these samples, including those from normal individuals, exhibited LD values of 600–850 U/L and aldolase values of 7.6–14.5 U/L; none of these results were included in the summary of LD or aldolase results. In contrast, CK values did not exhibit these artifactual elevations.

As shown in Table 1, CK-MB was fivefold greater in affected males than in unaffected males. The proportion of CK that was CK-MB (CK-MB/CK) also was increased in affected males, with six of the 10 values exceeding 3% (range 2.1–5.0% for all 10). The proportion of LD-1 was not increased. Carrier females showed no significant increases of muscle-origin enzymes or isoenzymes.

Serum CK, expressed as multiples of the upper limits of normal, decreased with age in EDMD (Figure 1). Results of the earlier studies of Family 1 by Emery and Dreifuss (2) and McKusick (9) are included. The correlation coefficient for the linear-regression line shown is 0.66 (P < 0.01). Because CK varies widely in children, the estimates of the upper limits of normal for children are less secure than those for adults. When the values for the two youngest individuals (T.F. and J.D.) were omitted from the linear-regression analysis, the correlation coefficient improved to 0.82 (P < 0.001).

Discussion

DNA analysis proved to be a powerful tool in identifying carrier females and was useful in confirming the diagnoses of asymptomatic affected males, but CK remains the analyst of choice for identifying males affected with EDMD. Results from Johnston and McKay (16) suggest that the CK activity concentration is within normal limits at birth in EDMD patients, and may still be so at age one year, but increases steadily thereafter. Our results suggest that CK peaks in childhood or adolescence and then declines slowly.

---

Table 1. Activity Concentrations of Muscle Enzymes in EDMD Families

<table>
<thead>
<tr>
<th>Affected males</th>
<th>CK, U/L</th>
<th>CK, MUL</th>
<th>CK-MB, U/L</th>
<th>LD, U/L</th>
<th>LD, MUL</th>
<th>LD-1, %</th>
<th>Aldolase, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K.Y.</td>
<td>356</td>
<td>1.75</td>
<td>7</td>
<td>393</td>
<td>0.87</td>
<td>26.2</td>
</tr>
<tr>
<td></td>
<td>H.Y.</td>
<td>589</td>
<td>2.89</td>
<td>23</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>S.C.</td>
<td>998</td>
<td>4.89</td>
<td>39</td>
<td>445</td>
<td>0.99</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>S.E.</td>
<td>473</td>
<td>2.32</td>
<td>23</td>
<td>505</td>
<td>1.12</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td>R.D.</td>
<td>1268, 782</td>
<td>6.21, 3.83</td>
<td>41, 38.2</td>
<td>500, 495</td>
<td>1.11, 1.10</td>
<td>24.8, 30.1</td>
</tr>
<tr>
<td></td>
<td>J.D.</td>
<td>449</td>
<td>1.21</td>
<td>15.8</td>
<td>495</td>
<td>0.85</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>T.F.</td>
<td>1523</td>
<td>4.12</td>
<td>62.8</td>
<td>799</td>
<td>1.14</td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>W.C.</td>
<td>294</td>
<td>1.44</td>
<td>&lt;8</td>
<td>316</td>
<td>0.70</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>B.C.</td>
<td>125</td>
<td>0.61</td>
<td>3.6</td>
<td>391</td>
<td>0.87</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>T.M.</td>
<td>413</td>
<td>2.02</td>
<td>9.0</td>
<td>401</td>
<td>0.89</td>
<td>—</td>
</tr>
</tbody>
</table>

Results (mean ± SE) for experimental groups

<table>
<thead>
<tr>
<th>Affected males</th>
<th>624.4 ± 128.0</th>
<th>2.63*</th>
<th>24.5 ± 6.0*</th>
<th>471.4 ± 43.3</th>
<th>0.95*</th>
<th>30.2 ± 1.2</th>
<th>11.9 ± 2.1*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10)</td>
<td>(10)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(7)</td>
<td>(9)</td>
</tr>
<tr>
<td>Obligate carriers</td>
<td>103.7 ± 10.2</td>
<td>0.49</td>
<td>1.5 ± 0.4</td>
<td>321.8 ± 11.6</td>
<td>0.69</td>
<td>34.3 ± 1.2</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Obligate + probable</td>
<td>100.5 ± 8.3</td>
<td>0.45</td>
<td>2.3 ± 0.4</td>
<td>332.1 ± 11.8</td>
<td>0.69</td>
<td>34.1 ± 0.9</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(33)</td>
<td>(33)</td>
<td>(23)</td>
<td>(28)</td>
<td>(28)</td>
<td>(19)</td>
<td>(30)</td>
</tr>
<tr>
<td>Normal males</td>
<td>136.3 ± 11.2</td>
<td>0.53</td>
<td>4.6 ± 1.1</td>
<td>399.6 ± 29.4</td>
<td>0.74</td>
<td>35.0 ± 1.2</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(21)</td>
<td>(21)</td>
<td>(17)</td>
<td>(17)</td>
<td>(17)</td>
<td>(15)</td>
<td>(15)</td>
</tr>
<tr>
<td>Normal females</td>
<td>92.9 ± 10.9</td>
<td>0.41</td>
<td>1.8 ± 0.3</td>
<td>306.9 ± 12.2</td>
<td>0.65</td>
<td>30.8 ± 2.6</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(20)</td>
<td>(19)</td>
<td>(16)</td>
<td>(16)</td>
<td>(9)</td>
<td>(15)</td>
</tr>
</tbody>
</table>

MUL, multiples of upper limit of normal. Unless otherwise specified, the upper limits were 204 U/L for CK and 450 U/L for LD. * Normal reference range is 2.5–4.0 U/L. Estimated upper limit of normal for this age (12 y) is 370 U/L for CK (n = 48), 580 U/L for LD (n = 49). Estimated upper limit of normal for this age (5 y) is 370 U/L for CK (n = 27), 700 U/L for LD (n = 25). Numbers in parentheses represent number of subjects in each group. * Significant at P < 0.001 vs unaffected males. Significant at P < 0.005 vs unaffected males.
Throughout adult life, Rotthauwe et al. (13) noted increases of CK in six younger affected males, but normal values in three men older than 40 years. A similar pattern of declining CK has been reported in DMD, both in affected males and obligate carrier females (29). Although LD and aldolase showed statistically significant increases, most individual values were not outside the normal reference interval, limiting the clinical usefulness of these tests. Despite reports of increased CK in 25% or more of carrier females (2, 18, 19), statistical analysis of our data revealed no significant differences from normal females. None of the muscle enzymes tested appeared useful for identifying carrier females.

The proportion of CK-MB has been reported to be normal in three sporadic cases of EDMD (5, 12). Given reports of autosomal dominant transmission of EDMD (30–35), which may represent a different but similar disease process, results from sporadic cases may not always be applicable to X-linked pedigrees. Changes in CK-MB have not been previously reported in X-linked pedigrees. The mildly increased mean of CK-MB in affected males is less than is generally reported in DMD (36), in which mean results of 12% have been reported in the early stages of the disease (37). The lack of increase of LD-1 suggests that there is not significant cardiac muscle damage. Rather, increased serum CK-MB may represent an increase of this isoenzyme in the dystrophic skeletal muscle, as has been reported for DMD (38–40).

This work was supported by a fellowship grant from the Muscular Dystrophy Association (M.G.B.) and a research grant from the Muscular Dystrophy Association (T.E.K.). We thank Mrs. Lynn McCutcheon and Mrs. Betty Roberts for secretarial assistance.

References