Muscle Creatine Phosphokinase in Primary Myopathies* (32929)

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In mammalian tissues creatine phosphokinase (CPK) activity is highest in normal skeletal muscle (1–9). Studies of muscle CPK in healthy human subjects and in patients with myopathy or neuropathy have been reported (8–12) but the observations have been limited in number and assay techniques have not been uniform. The data herein presented enlarge our experience with measurements of this enzyme in human muscle in health and in myopathy. They demonstrate that if fresh samples are kept cold and analyzed promptly the addition of a sulfhydryl group to the assay mixture does not significantly augment the activity of the enzyme. They also establish that in pseudohypertrophic muscular dystrophy low CPK activity in relation to the wet weight of tissue is associated with normal CPK activity in terms of the supernate nitrogen of the assay preparation.

Materials and Methods. The CPK activity has been measured in enzyme preparations of biopsies of skeletal muscle from control subjects or patients with various forms of muscular dystrophy, myotonia dystrophica or myositis and correlated with levels of nitrogen in the supernate of such muscle homogenates.

Cylindrical samples of deltoid or gastrocnemius muscle, approximating 3 mm in diameter and 5 to 10 mm in length, obtained by means of an electric drill fitted with a hollow cylindrical cutting head (13), were immediately refrigerated at 0–4°C prior to measurement of CPK activity in the next 1–4 hours. The samples were comminuted in 0.154 *M* KCl in water in a Potter-Elvehjem homogenizer. The suspension was then centrifuged at 39.5*g* for 55 min in a model L Spinco preparative ultracentrifuge and a portion of the supernate was analyzed for nitrogen by the micro-Kjeldahl technique. The CPK activity in a sample of the supernate was measured via the single enzyme backward reaction: ($C + ATP \rightleftharpoons CP + ADP$) by the method of Noda *et al.* (14) with slight modifications. Thus, ATP concentration was increased eightfold and the creatine concentration was doubled. These modifications yielded rates that were first order with respect to enzyme activity. In about one half of the measurements mercaptoethanol in 7×10^{-4} molar concentration as a source of sulfhydryl groups was added to duplicates of the assay mixtures. Results of assays are stated in terms of μmoles of creatine phosphate [measured as phosphate (15)], produced per gm of wet muscle, or per mg of N in the supernate, per min.

Results. Under the experimental conditions cited, viz., prompt analysis of refrigerated fresh biopsy specimens, the addition of mercaptoethanol was not associated with a statistically significant difference in the CPK activity of the 27 muscle samples studied (Table I). Findings in muscle from control subjects and patients with pseudohypertrophic, limb girdle, and facio-scapulo-humeral muscular dystrophy, myotonia dystrophica, or myositis are presented in Table II.

In terms of wet weight of tissue, muscle CPK significantly lower than that of control subjects was recorded only in patients with pseudohypertrophic muscular dystrophy. However, in relation to supernate nitrogen, muscle CPK activity was within the control range in the pseudohypertrophic form of dystrophy (Table II). Again, as in Table I, it is evident that results on assay were not augmented by mercaptoethanol.

Discussion. Measurements of CPK activity in normal or diseased human muscle with or without added sulfhydryl compounds are limited (Table III). Thus, Okinaka *et al.* (10)

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TABLE I. CPK Activity in Fresh, Refrigerated Human Muscle Assayed without and with the Addition of Mercaptoethanol.

	Muscle CPK[a]	
	μmoles/gm per min without –SH	μmoles/gm per min with –SH
	$X \pm$ SD (no.)	$X \pm$ SD (no.)
Control subjects	740 ± 62 (11)	779 ± 101 (11)
Myopathies[b]	691 ± 251 (33)	737 ± 261 (16)

[a] Differences in assay results obtained without and with addition of sulfhydryl compound are not statistically significant.

[b] Diagnoses in the 33 patients are indicated in Table II.

reported that in normal skeletal muscle the addition of a sulfhydryl compound to the assay preparation had no significant effect upon CPK activity in two samples of normal muscle or in four biopsy specimens from patients with muscular dystrophy or myositis. On the other hand the data of Kar and Pearson (11) based on analysis of one sample of human thigh muscle indicate that the addition of a sulfhydryl compound did increase CPK activity some threefold. However their specimen, unlike those of Okinaka and ours, was obtained at autopsy 10 hours after death. It would appear therefore that prompt assay of refrigerated samples of muscle obtained at biopsy yields values which are not significantly increased by the addition of a sulfhydryl compound to the assay mixture.

In the studies of Okinaka *et al.* (10) muscle CPK activity in muscular dystrophy and in myositis was definitely lower than that in samples from healthy subjects. Qualitatively, our findings are in agreement with those of Okinaka *et al.* but their control values were about fourfold higher, while those obtained with samples of muscle affected by dystrophy or myositis were about the same as ours. We have no explanation for differences in the control values. They may be related to modifications of the experimental conditions, since the assay technique of Noda *et al.* (14) based on the one enzyme backward reaction was employed in both laboratories.

The values for normal human muscle reported by Okinaka and his co-workers as well as ours are in turn higher than those of Kar and Pearson (11). However, in addition to studying autopsy material, the latter used the three enzyme reaction of Tanzer and Gilvarg (3). In this assay pyruvic kinase is employed in a second enzyme reaction to produce pyruvate from added phosphoenolpyruvate and the ADP formed in the first enzyme reaction; in a third enzyme reaction the pyruvate participates in the conversion of added NADH to NAD under the influence of lactic acid dehydrogenase. Since this assay

TABLE II. CPK Activity in Human Muscle in Terms of Wet Weight and Supernate Nitrogen.

	Muscle CPK			
	μmoles/gm per min		μmoles/mg of N/min	
	without –SH	with –SH	without –SH	with –SH
	$X \pm$ SD (no.)	$X \pm$ SD (no.)	$X \pm$ SD (no.)	$X \pm$ SD (no.)
Controls[a]	740 ± 62 (11)	779 ± 101 (11)	87 ± 40 (11)	92 ± 47 (11)
Dystrophy				
PHMD[a]	493 ± 131 (15)	556 ± 160 (9)	92 ± 38 (11)	103 ± 47 (8)
Limb girdle	722 ± 279 (5)	1026 ± 250 (2)	108 ± 19 (4)	113 ± 14 (2)
FSH	874 ± 158 (7)	903 ± 157 (3)	92 ± 23 (7)	83 ± 9 (3)
Myotonia dystrophica	939 ± 168 (4)		132 ± 20 (4)	
Myositis	960 ± 117 (2)	1008 ± 129 (2)	132 ± 63 (2)	140 ± 67 (2)

[a] Differences between values in controls and in pseudohypertrophic muscular dystrophy (PHMD) are statistically significant in terms of μmoles/gm per min but not in terms of μmoles /mg of N/min; the numbers of observations in the limb girdle, facio-scapulo-humeral (FSH), myotonia dystrophica, and myositis groups are too few for statistical evaluation.

TABLE III. Reports of CPK in Human Muscle.

Ref.	Reaction and end point	Added –SH	Diagnosis	Human muscle	CPK: μmoles/gm per min wet tissue	CPK: μmoles/mg of protein[b]/min (× 10^{-4})	CPK: μmoles/gm of N[b]/min
Okinaka *et al.* (10)	Backward (creatine phosphate as phosphate)	yes[c]	normal	quadriceps	2617[a]		
			normal	gastrocnemius	3200[a]		
			PHMD	quadriceps	428[a]		
			PHMD	quadriceps	195[a]		
			myositis	quadriceps	582[a]		
			dermatomyositis	quadriceps	447[a]		
Kar and Pearson (11)	Backward (decrease in NADH)	yes[d]	10 hours after death	thigh	16.4[d]		
Colombo *et al.* (8)	Backward (decrease in NADH)	no	normal	psoas			2383
				rectus abd			1673
Hess *et al.* (9)	Backward (decrease in NADH)	no	normal	10 muscles at surgery		18,405	

[a] Reported in original article as μm/g/hour, therefore 60-fold higher.
[b] Protein or nitrogen in supernate.
[c] Addition of –SH had no effect on muscle CPK.
[d] Addition of –SH produced threefold increase in CPK activity from 5.3 to 16.4 ''U/g of wet tissue,'' but 16.4 probably means 16,400/gm per min [see original report (11)].

technique applied to serum also yields values lower than those obtained with the one enzyme backward reaction (8, 10), such lower values presumably reflect the presence of unidentified rate limiting variables in the three-enzyme assay. This possibility is partially supported by the low values for muscle CPK reported by Columbo (8) and by Hess (9) and their colleagues (Table III) who employed the three enzyme assay system for studies of surgical rather than autopsy material but did not add a sulfhydryl compound. Hence further studies of the precision and the limitations of CPK assays in muscle are needed.

Our data suggest that in terms of wet weight of the biopsy sample low values for muscle CPK do but need not occur in pseudohypertrophic muscular dystrophy. However, when such decreases in muscle CPK in terms of weight of wet tissue are present, CPK activity is found to be normal in relation to the nitrogen in the supernate in the assay sample. Thus, in limb girdle or facio-scapulo-humeral muscular dystrophy, in myotonia dystrophica and in myositis, muscle CPK activity appears to be normal or perhaps high (our observations are limited in number) in terms of the wet weight of the sample. These data are consonant with the marked loss of muscle fibers, the presence of fat, fibrosis, edema, and cellular infiltrates in large amounts in pseudohypertrophic muscular dystrophy, and in keeping with their relative infrequency in limb girdle and facio-scapulo-humeral dystrophy, myotonia dystrophica, and myositis (16).

Our findings suggest that in pseudohypertrophic muscle enzyme activity is normal in relation to supernate nitrogen. Hence, even though the quantity of CPK in muscle is decreased in pseudohypertrophic muscular dystrophy, the activity of whatever CPK is present remains within the normal range.

Summary and Conclusions. In terms of wet weight of the biopsy sample, muscle creatine phosphokinase (CPK) may be decreased in skeletal muscle from patients with pseudohypertrophic muscular dystrophy (PHMD); however, despite such decreases, CPK activity in terms of nitrogen in the supernate of the assay sample remains within the normal range. Hence the low values, when present, result in all probability from the increased fat content of muscle. Muscle CPK per unit of wet weight or in relation to supernate nitrogen in the assay sample was not low in a limited number of observations in limb girdle and facio-scapulo-humeral muscular dystrophy; this also appeared to be so in the few examples of myotonia dystrophica and of polymyositis examined by the above technics. The addition of a sulfhydryl compound to the assay mixture did not produce a statistically significant difference in the CPK activity of skeletal muscle obtained at biopsy which was refrigerated and assayed promptly. Quantitative differences in the reports of assays of muscle CPK activity by methods in current use are cited.

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