Abnormal protein synthesis in facioscapulohumeral muscular dystrophy

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■ Recently we have observed that muscle polyribosomes obtained from patients with Duchenne muscular dystrophy (DMD) synthesize abnormally large amounts of collagen when combined with soluble enzymes derived from the same patient's muscle.[1] Increased protein synthesis in the carrier state of DMD has also been observed and has proved useful in the detection of heterozygotes.[2]

In this study, we tested four biochemical parameters in skeletal muscle from patients with facioscapulohumeral (FSH) muscular dystrophy: (1) total ribosome concentration, (2) ribosome distribution, (3) in vitro amino acid incorporation by fractionated ribosomes, and (4) in vitro collagen synthesis. Because three of these parameters are abnormal in patients and two are abnormal in carriers of DMD,[1-3] we wanted to know if similar disturbances in ribosomal protein synthesis are found in FSH muscular dystrophy.

Material and methods

Eight patients with FSH muscular dystrophy and five normal controls matched for sex and age were examined. Muscle samples of patients and controls were obtained from the left vastus lateralis. Technical details about the muscle biopsy, the procedure for the preparation of the muscle extracts, and the evaluation of ribosomal protein synthesis were presented in previous papers.[1,4]

The specimens for light microscopy were fixed in Susa's fixative, and longitudinal and transverse sections were stained with hematoxylin and eosin, Mallory's trichrome, phosphotungstic acid-hematoxylin, and periodic acid-Schiff reagent.

Determination of noncollagen protein in the muscle homogenate was done by the method of Lowry and associates[5] using bovine serum albumin as standard. A statistical analysis of the data was done by estimation of Fisher's t coefficient.

The method of Hughes,[6] as modified by Bray and Ferrendelli,[7] was used for the determination of serum creatine phosphokinase (CPK) levels.

Results

All the patients had a family history of the disease, which suggests autosomal dominant inheritance. Although all had the typical symptoms of facial weakness, they seemed to be clearly separable into two groups depending on how far the condition had progressed. Four of them were in an *early stage* of the disease since only the facial muscles were involved; furthermore, the deltoid muscle biopsy gave a normal histologic picture (cases 1 through 4). The other four (cases 5 through 8) were in an *advanced stage*, with weakness and atrophy of

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This study was supported in part by the Muscular Dystrophy Association of America, Inc., the National Institutes of Health, and USPHS Clinical Research Center grant M01-FR-59 for patient services.

Received for publication January 28, 1972.

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the shoulder and pelvic muscles; on histologic examination, biopsies of their deltoid muscles showed dystrophic changes dominated by variation in myofiber size (table 1). Serum CPK level was moderately elevated in six patients (three in early and three in late stages) and normal in two; thus, the elevated CPK level was not useful in grouping the patients into early or advanced stages.

Homogenates of the biopsied muscle were used to prepare ribosomes, which were separated into several classes by sucrose density gradient centrifugation. The analysis of their distribution and activity was done as previously reported for patients with DMD.[1]

1) *Ribosome content.* As seen in table 2, the concentration of ribosomes in all the classes studied was the same both in patients and controls when expressed in terms of the amount of noncollagen protein. The noncollagen protein content itself was also normal in the patients, being 67.5 ± 22.8 (S.D.) mg of protein per gram of wet muscle compared with 75.0 ± 8 (S.D.) mg per gram in the controls.

2) *Distribution of ribosomes.* Previously we observed an abnormal ribosome pattern in patients with DMD, which consisted of a sharp peak of monomeric ribosomes with very few polyribosomes. Controls showed a smaller and broader monomeric peak with many more polyribosomes on the slowly descending shoulder on the right (heavy) side of the major peak (figure 1). Muscle ribosomes from seven of the eight patients showed normal distributions on sucrose density gradients. The solid line in figure 1 shows the distribution of ribosomes from the deltoid muscle in a typical control, while figure 2 illustrates the very similar ribosome pattern from a patient in an early stage of the disease. Of the eight FSH patients, only one (case 5) showed an abnormal pattern with an increase in monomeric ribosomes.

3) *Amino acid incorporation in the early stage.* After separation of the ribosomes into 20 fractions by sucrose density gradient centrifugation, the incorporation of a mixture of C^{14}-labeled amino acids (Schwartz Bioresearch 3122-08) in the presence of added soluble enzymes from the same subject was used as a measure of protein synthesis.[1,4] The specific activity of dystrophic polyribosomes for incorporating amino acids was significantly higher than normal only in the early stage of FSH muscular dystrophy (table 3 and figure 2). The average value for these polyribosomes was 158 ± 94.8 cpm per microgram of polyribosomes compared with an average of 50 ± 18 cpm per microgram for the controls. The specific activ-

TABLE 1
HISTOLOGIC CHANGES IN THE ADVANCED STAGE OF FSH

Case No.	*Variation in myofiber size*	*Increase in sarcolemmal nuclei*	*Regenerating myofibers*	*Degenerating myofibers*	*Increase in endomysial connective tissue*
5	+	–	+	–	–
6	+	–	+	+	–
7	+	–	–	–	–
8	+	–	–	+	–

TABLE 2
RIBOSOME CONTENT OF MUSCLE

		*Ribosome content (μg/mg of protein)**			
	Noncollagen protein (mg/gm of wet muscle)	*Total ribosomes*	*Major ribosome fraction*	*Free ribosomes*	*Reextracted ribosomes*
Patients (8)	67.5 ± 22.8	7.1 ± 2.5	4.0 ± 2.1	1.2 ± 0.58	1.9 ± 1.08
Controls (5)	75.0 ± 8	9.2 ± 1	5.4 ± 1.5	1.6 ± 0.6	2.2 ± 1.3
t value	0.70	1.76	1.29	1.16	0.45
p value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

*The major ribosome fraction was obtained by extraction of an initial 122,000 × g pellet with detergent.[1] The free ribosomes were obtained by recentrifugation of the initial high-speed supernatant fraction at 150,000 × g for two hours. Further extractions of the 122,000 × g pellet with a doubled concentration of detergent produced the reextracted ribosome fraction.

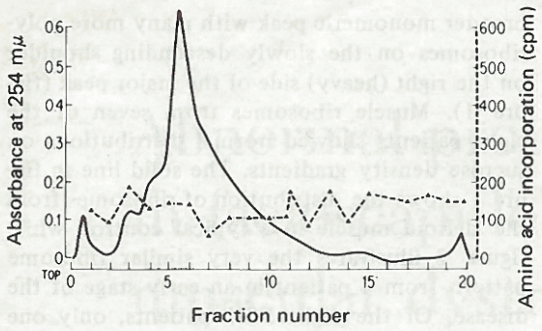

Figure 1. Sucrose density gradient analysis of ribosomes from the deltoid muscle of a 10 year old control. The solid line describes the absorbance measured continuously at 254 mμ; the broken line indicates the amino acid incorporating activity of isolated fractions determined after separation on the gradient. The gradient was layered with 105 μg of the major ribosome fraction. The control's soluble enzyme (86 μg) was added to each fraction separated from the gradient.

ity of the dystrophic monomeric ribosomes was significantly low, 5.4 ± 1.7 cpm per microgram; the normal value was 15.5 ± 4 cpm per microgram. We have found that for DMD patients or carriers, the activities of the polyribosomes compared with those of the monomeric ribosomes are very useful in the detection of both the diseased and the carrier state. For example,

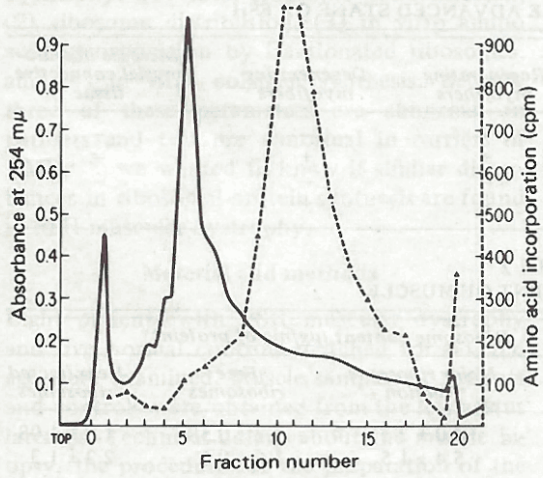

Figure 2. Sucrose density gradient analysis of ribosomes from the deltoid muscle of a four year old patient (case 1) in the early stage of FSH muscular dystrophy. The gradient was layered with 128 μg of the major ribosome fraction. The amount of the dystrophic soluble enzyme used was 90 μg.

patients with DMD have ratios of about 33; carriers, about 6 to 17; and controls, about 3.3.[1,2] Similarly, in the early stage of FSH muscular dystrophy, the ratio of the specific activities of the polyribosomes to the monomeric ribosomes showed highly significant differences between the dystrophic and control groups, i.e., 27.2 ± 8 for the patients compared with 3.2 ± 1 for the controls (table 3).

The specific activity of the ribosomes isolated from the vastus lateralis muscle was similar to that of ribosomes from the deltoid muscle of two patients; however, the values of protein synthesis for monomeric ribosomes and polyribosomes were even higher (figures 3 and 4).

4) *Amino acid incorporation in the advanced stage.* The advanced stage of FSH muscular dystrophy was characterized by rather low values of protein synthesis for the dystrophic polyribosomes. However, the average specific activity of 35.2 ± 13.1 cpm per microgram of ribosomes was not significantly decreased when compared with that of the controls (table 4 and figure 5). The monomeric ribosomes maintained a significantly low specific activity of 9.3 ± 2.3 cpm per microgram of ribosomes, which, while low, was not so striking as in the early stage of the disease. The ratio of the specific activities of the polyribosomes to the monomeric ribosomes, which is high in DMD in all stages, was very close to the values of the controls.

5) *In vitro synthesis of collagen.* Collagen formation by the sedimented polyribosomes from our eight patients was within normal

TABLE 3
AMINO ACID INCORPORATION OF POLYRIBOSOMES AND MONOMERIC RIBOSOMES* IN THE EARLY STAGE

Case No.	*A. Monomeric ribosomes*	*B. Fractionated and sedimented polyribosomes*	*C. Ratio B/A*
1	5.2	164.0	31.5
2	8.0	290.0	36.2
3	4.0	88.1	22.0
4	4.7	90.0	19.1
Mean	5.4 ± 1.7	158.0 ± 94.8	27.2 ± 8
Controls (5)	15.5 ± 4	50 ± 18	3.2 ± 1
t value	4.6	−2.5	−6.7
p value	< 0.01	< 0.05	< 0.01

*Measured in counts per minute per microgram of ribosomes.

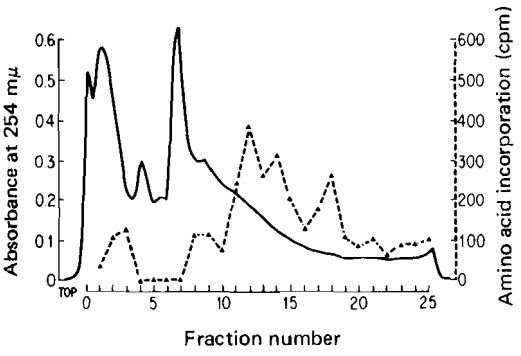

Figure 3. Sucrose density gradient analysis of ribosomes from the deltoid muscle of an 11 year old patient (case 3) in the early stage of FSH muscular dystrophy. The gradient was layered with 123 μg of the major ribosome fraction. The amount of the dystrophic soluble enzyme used was 91 μg.

limits, as shown in table 5. The average value of 16.2 ± 12.2 cpm per microgram of ribosomes for the patients represents 8.4 percent of the total specific activity of this class of polyribosomes. In this respect, the difference of FSH versus DMD patients is very dramatic, since in DMD 85 to 100 percent of the protein synthesized by these ribosomes represents collagen.[1] Furthermore, collagen is not synthesized by the fractionated ribosomes (figures 5 and 6). Figure 5 shows the protein synthetic activity of ribosomes separated by sucrose density gradient centrifugation; figure 6 is identical

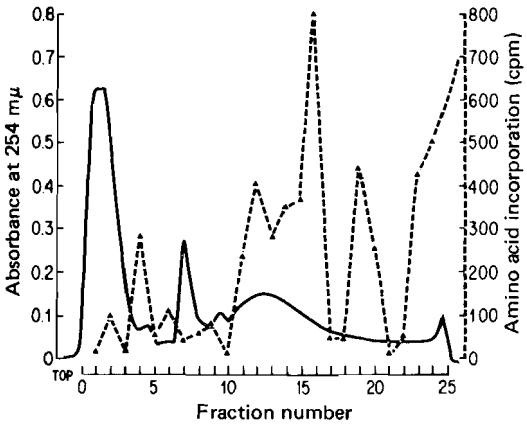

Figure 4. Sucrose density gradient analysis of ribosomes from the vastus lateralis muscle of the same patient as in figure 3. The gradient was layered with 116 μg of the major ribosome fraction. The amount of dystrophic soluble enzyme used was 88 μg.

except that collagenase has been used to make trichloroacetic acid-soluble any collagen protein labeled in vitro. Collagen synthesis is detected by a lowering of incorporation, as shown in figure 6 compared with figure 5. Since both incorporation profiles are approximately the same, within the errors of the experiment, we conclude that the material from this patient, who is in an advanced stage of the disease, does not synthesize appreciable amounts of collagen. A similar experiment (not shown here) using material from a patient in an early stage of the disease (case 2) also showed normal levels of collagen labeling on sucrose density gradient analysis. On the other hand, we showed previously that in DMD, 20 to 40 percent of the amino acids were incorporated into collagen by the sucrose density gradient fractions of the dystrophic ribosomes.

Discussion

Facioscapulohumeral muscular dystrophy differs in several ways from DMD. The inheritance of FSH muscular dystrophy is autosomal dominant while that of the Duchenne type is X-linked recessive. Furthermore, FSH muscular dystrophy occurs less frequently, and as the name indicates, the muscles of the face and shoulders are initially involved. Progression is slower than in DMD, and this is reflected by normal or only moderately high levels of serum CPK.

A study of ribosomal protein synthesis in muscle extracts from patients with FSH muscular dystrophy has not been previously performed. Our results, summarized in table 6,

TABLE 4
AMINO ACID INCORPORATION OF POLYRIBOSOMES AND MONOMERIC RIBOSOMES* IN THE ADVANCED STAGE

Case No.	*A. Monomeric ribosomes*	*B. Fractionated and sedimented polyribosomes*	*C. Ratio B/A*
5	12.0	23.3	1.9
6	8.6	54.0	6.3
7	6.5	31.0	4.7
8	10.1	32.6	3.2
Mean	9.3 ± 2.3	35.2 ± 13.1	4.0 ± 1.9
Controls (5)	15.5 ± 4	50 ± 18	3.2 ± 1
t value	2.7	1.37	− 0.84
p value	< 0.05	> 0.05	> 0.05

*Measured in counts per minute per microgram of ribosomes.

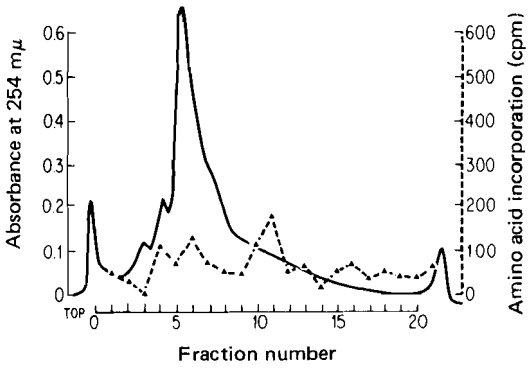

Figure 5. Sucrose density gradient analysis of ribosomes from the deltoid muscle of a 34 year old patient (case 8) in the advanced stage of FSH muscular dystrophy. The gradient was layered with 72 μg of the major ribosome fraction. The amount of dystrophic soluble enzyme used was 110 μg.

show that protein synthesis in FSH deviates from normal by an increased rate of synthesis during the early stage of the disease and by a decrease in protein synthesis in monomeric ribosomes at all stages of the disease. Table 6 also shows some differences with protein synthesis in DMD. The concentration of noncollagen protein in the sedimented ribosomes was within normal values even in the advanced stage of the disease (tables 5 and 6), while all our patients with DMD had low values. The distribution of the muscle ribosomes on sucrose density gradients produced the distinctive Duchenne profile only in one patient; the other seven had a normal pattern. The specific activity of dystrophic monomeric ribosomes for incorporating amino acids in vitro was significantly low throughout the whole course of FSH muscular dystrophy. The latter change might be caused by any number of factors, including a possible disorder in the initiation of protein synthesis.

An increased activity of dystrophic fractionated polyribosomes for incorporating amino acids in vitro was significantly high only in the early stage of the disease. The sedimented polyribosomes, which in DMD incorporate amino acids mostly into collagen, have normal activity in FSH muscular dystrophy. The absence of any increase in collagen synthesis is the major difference between FSH muscular dystrophy and DMD (tables 5 and 6). Perhaps this biochemical feature is responsible for the benign course of the disease. Thus, the high amino acid incorporation of the FSH polyribosomes reflects the synthesis of noncollagen, perhaps contractile protein, consistent with muscle regeneration. The most active ribosomes are contained in the fractions 10 through 15 (figures 2, 3 and 4). According to the experiments of Heywood and Rich[8] with chick embryo muscle, these fractions could synthesize actin or tropomyosin.

To illustrate correlations between amino acid incorporation of FSH dystrophic ribosomes, clinical findings, serum CPK, and histologic findings in muscle, the data are summarized in table 7. Patients in the early stage

TABLE 5
PROTEIN SYNTHESIS OF SEDIMENTED MUSCLE POLYRIBOSOMES*

Case No.	*Noncollagen protein*	*Collagen protein*
1	160	24
2	310	31
3	200	0
4	120	30
5	272	0
6	110	20
7	150	15
8	86	10
Mean	176 ± 79.5	16.2 ± 12.2
Controls (5)	200 ± 75	30 ± 20
t value	0.30	1.76
p value	>0.05	> 0.05

*Measured in counts per minute per microgram of ribosomes.

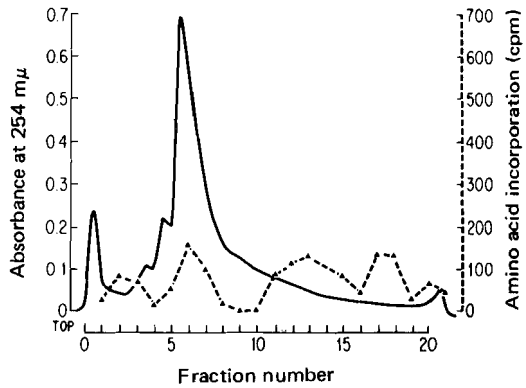

Figure 6. Sucrose density gradient analysis of ribosomes from the deltoid muscle of the same patient as in figure 5. Collagenase test was performed on these ribosomes. The amount of ribosomes layered on the gradient and the amount of dystrophic enzyme were the same as in figure 5.

(cases 1 through 4) show changes in ribosomal protein synthesis of the deltoid muscle, yet the results of the routine histologic examinations are normal. Proliferation of endomysial connective tissue, a typical finding in DMD, even in the preclinical stage,[9] was not seen even in the advanced stage of FSH muscular dystrophy (table 1). Furthermore, the serum CPK level was elevated in three FSH patients and normal in one. This emphasizes the sensitivity of using protein synthesis for detecting early muscle changes which may not always be detected by the usual histologic methods or changes in serum CPK. It is possible, however, that more refined technics such as histochemistry and electron microscopy might be developed to where early stage abnormalities could be seen. The detection of DMD carriers presents the same sort of problem since some true carriers fail to show changes in CPK or histologic findings in muscle but can be detected by changes in protein synthesis.[2] A patient in an early stage of FSH muscular dystrophy showed increased protein synthesis not only in extracts derived from deltoid muscle but also in those from the vastus lateralis, indicating the extension of the biochemical disorder even though clinical involvement of the latter muscle is seen only in advanced stages. The advanced stage (cases 5 through 8) was clinically characterized by the involvement of girdle muscles as well as facial muscles and histologic abnormalities of a dystrophic type (variation in myofiber size and degenerating and regenerating myofibers) with moderately elevated CPK levels in three cases. Nevertheless, amino acid incorporation of the polyribosomes was decreased in three patients and within the normal range in one. We could

TABLE 6
COMPARISON OF PROTEIN SYNTHESIS IN DUCHENNE AND FSH MUSCULAR DYSTROPHY

	Ribosomes				*Protein synthesized on sedimented ribosomes*	
			Amino acid incorporation			
Muscular dystrophy	*Concentration*	*Distribution on sucrose gradient*	*Monomeric*	*Fractionated*	*Noncollagen*	*Collagen*
Duchenne	Normal	Abnormal	Low	High	Low	High
FSH						
Early	Normal	Normal	Low	High	Normal	Normal
Late	Normal	Normal	Low	Normal	Normal	Normal

TABLE 7
CORRELATIONS BETWEEN AMINO ACID INCORPORATION OF POLYRIBOSOMES AND MONOMERIC RIBOSOMES, CLINICAL FINDINGS, SERUM CPK, AND HISTOLOGIC FINDINGS IN MUSCLE

Case No.	*Age (years)*	*Progression (years)*	*Serum CPK*	*Histologic findings in muscle, deltoid*	*Amino acid incorporation compared with normal*	
					Monomeric	*Polyribosomes*
*Early stage**						
1	4	1	High	Normal	Low	High
2	8	4	Normal	Normal	Low	High
3	11	8	High	Normal	Low	High
				Normal (vastus lateralis)	Low	High
4	13	10	High	Normal	Low	High
Late stage†						
5	14	11	High	Dystrophic	Low	Low
6	15	8	High	Dystrophic	Low	Low
7	16	12	High	Dystrophic	Low	Normal
				Dystrophic (vastus lateralis)	Low	High
8	34	22	Normal	Dystrophic	Low	Low

*Early stage = weakness of facial muscles.
†Late stage = weakness of the facial, shoulder and pelvic muscles.

not establish any correlation between the ribosomal protein synthesis and the histologic evidence of muscle regeneration.

The increase in protein synthesis seen in the early stage of FSH may only reflect an attempt at muscle regeneration that is secondary to and triggered by an undetermined genetic defect. In this view, the primary defect would exist throughout the course of the disease, but muscle regeneration, as evidenced by protein synthesis, would fail in late stages. Alternatively, the uncoordinated synthesis of a single structural protein such as actin might reflect the primary genetic defect and lead to a lowering of the synthesis of other proteins essential to normal muscle function. Conceivably, this could cause an excessive turnover of the contractile proteins or faulty arrangement of the proteins in the sarcomere. To test this view, we are attempting to identify the nature of the protein or proteins synthesized in large amounts in the early stage of the disease.

Summary

The ribosome content and distribution on sucrose density gradients of polyribosomes from deltoid muscle extracts were normal in seven of eight patients with inherited facioscapulohumeral muscular dystrophy. Incorporation of a mixture of radioactive amino acids by polyribosomes showed high values of noncollagen protein (threefold increase) only in the early stage (four patients), while in the advanced stage (four patients), low or normal values were noted. Unlike patients with Duchenne muscular dystrophy, all eight FSH patients had normal in vitro collagen synthesis.

Acknowledgments

We thank Drs. W. F. McCormick and S. Schochet for interpreting the histologic findings in muscle.

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