

Distinction between Duchenne and other muscular dystrophies by ribosomal protein synthesis

VICTOR IONASESCU

Department of Pediatrics, University Hospitals, Iowa City, Iowa 52242, USA

Summary. Ribosome concentration, ribosome distribution on sucrose density gradients, and *in-vitro* ribosomal amino-acid incorporation (noncollagen and collagen synthesis) were studied in muscle biopsy samples obtained from 30 patients with Duchenne muscular dystrophy, seven patients with Becker muscular dystrophy, and 10 with facioscapulohumeral muscular dystrophy. Ribosome concentration was normal in Duchenne and facioscapulohumeral and decreased in Becker muscular dystrophy. Distribution of ribosomes in sucrose density gradients showed abnormalities (sharp monosomal peak and fewer polyribosomes) only in Duchenne muscular dystrophy and was normal in the other two types. *In-vitro* amino-acid incorporation of ribosomes in Duchenne muscular dystrophy revealed high collagen and low noncollagen synthesis of the heavy polyribosomes. This abnormality is controlled by an undetermined enzymatic factor belonging to the soluble enzyme fraction. Supplementation of the dystrophic heavy polyribosomes with normal soluble enzymes restored the synthesis of collagen to that of the controls. Heavy polyribosomes extracted from normals or from carriers produce proportionately more collagen in the presence of soluble enzyme fraction from Duchenne muscular dystrophy than in the presence of their homologous enzymes. In Becker muscular dystrophy, both noncollagen and collagen synthesis of the heavy polyribosomes were increased, under the influence of ribosomal factors. The different protein synthesis in Duchenne and Becker muscular dystrophies suggests that these conditions are non-allelic. In facioscapulohumeral muscular dystrophy the changes in protein synthesis occurred only in the early stage of the disease and consisted of increased noncollagen synthesis of the light polyribosomes, while the heavy polyribosomes had normal activity including collagen synthesis. This reaction was controlled by ribosomal factors.

Our previous studies (Ionasescu, Zellweger, and Conway, 1971a) showed a higher protein synthesis particularly of collagen in the polyribosomes isolated from patients with Duchenne muscular dystrophy (MD). We also found an increased protein synthesis in the carriers of the disease which has proved useful in the detection of heterozygotes (Ionasescu *et al*, 1971a; 1973b). We then postulated whether this disorder in protein synthesis is specific for Duchenne

MD or might be found in other types of MD. In an attempt to answer this question, we have tested four biochemical parameters in skeletal muscle from patients with Duchenne, Becker, and facioscapulohumeral (FSH) MD. These parameters were (1) ribosome content, (2) ribosome distribution, (3) *in-vitro* aminoacid incorporation by different classes of ribosomes (total protein synthesis), (4) *in-vitro* collagen synthesis. Preliminary reports on changes in ribosomal activity in FSH and Becker MD have been previously published (Ionasescu *et al*, 1972; 1973a).

Material and methods

Thirty patients with Duchenne, seven with Becker, 10 with FSH MD, and 15 normal controls matched for sex and age were examined. Muscle samples of patients with Duchenne and Becker MD and 10 controls were obtained by biopsy of the left vastus lateralis. Muscle samples from the patients with FSH MD and five controls were obtained by biopsy of the left deltoid.

Technical details about the muscle biopsy, the procedure for the preparation of the muscle extracts and the evaluation of ribosomal protein synthesis were previously reported (Ionasescu *et al*, 1971a and b). The assay for collagen synthesis represented the difference in the trichloroacetic acid precipitable radioactivity in the absence and presence of purified *Clostridium histolyticum* collagenase (Worthington Biochemical Corp). Determination of the noncollagen protein in the muscle homogenate was done by the method of Lowry *et al* (1951) using bovine serum albumin as standard.

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

Results

Ribosome content. In Duchenne MD the content of ribosomes in all of the classes studied reached the upper limit of the normal values, when expressed in terms of the amount of noncollagen protein. The noncollagen protein of the muscle, however, was significantly decreased when compared with the values obtained from our controls. This suggests a defect in the function of these ribosomes. In Becker MD, a significant decrease in the concentration of the major ribosome fraction and free ribosomes was found, while the content of non-

collagen protein of muscle was normal. In FSH MD, the concentration of ribosomes and non-collagen protein content of muscle showed normal values (Table I).

Distribution of ribosomes on sucrose density gradients. An abnormal pattern, consisting of a sharp peak of monomeric ribosomes with very few polyribosomes was noted in ribosomes extracted from most patients with Duchenne MD (Fig. 1). Controls showed a smaller and broader monomeric peak with many more polyribosomes (Fig. 2). Normal distribution of muscle ribosomes isolated from patients with Becker and FSH MD was observed (Figs. 3 and 4).

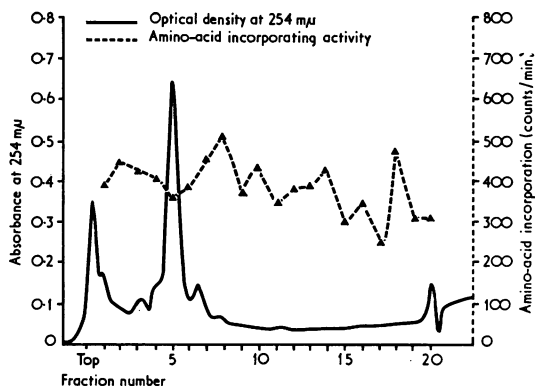


Fig. 1. Sucrose density gradient analysis of polyribosomes from muscle vastus lateralis of a 4-year-old patient with Duchenne MD (patient 5). Amino-acid incorporation was performed on isolated fractions after separation on the gradient. The gradient was layered with 110 μ g of the major ribosomal fraction. The amount of the dystrophic soluble enzyme used was 150 μ g per tube.

TABLE I
RIBOSOME CONTENT OF DYSTROPHIC MUSCLE

MD Type	Noncollagen Protein (mg/g muscle)	Ribosome Content (μ g/mg protein)*			
		Total Ribosomes	Major Ribosome Fraction	Free Ribosomes	Re-extracted Ribosomes
Duchenne (n = 30)	31.9 \pm 9.1	14.5 \pm 8.3	8.6 \pm 3.7	2.5 \pm 1.7	3.4 \pm 2.2
Controls (n = 10)	59.1 \pm 11.3	11.4 \pm 2.4	7.4 \pm 1.5	1.7 \pm 0.9	2.3 \pm 1.2
t	-7.5	1.7	1.0	1.4	1.4
p	< 0.01	> 0.05	> 0.05	> 0.05	> 0.05
Becker (n = 7)	64.2 \pm 9	5.6 \pm 0.9	3.4 \pm 1.1	0.9 \pm 0.2	1.3 \pm 0.5
Controls (n = 10)	59.1 \pm 11.3	11.4 \pm 2.4	7.4 \pm 1.5	1.7 \pm 0.9	2.3 \pm 1.2
t	1.0	-6.0	-6.0	-2.3	-2.0
p	> 0.05	< 0.01	< 0.01	< 0.05	> 0.05
FSH (n = 10)	69.1 \pm 20.3	7.5 \pm 2.9	4.6 \pm 2.3	1.1 \pm 0.7	1.8 \pm 1.2
Controls (n = 5)	75.0 \pm 8	9.2 \pm 1	5.4 \pm 1.5	1.6 \pm 0.6	2.2 \pm 1.3
t	-0.6	-1.2	-0.7	-1.4	-0.6
p	> 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. The free ribosomes were obtained by re-centrifugation 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 re-extracted ribosome fraction.

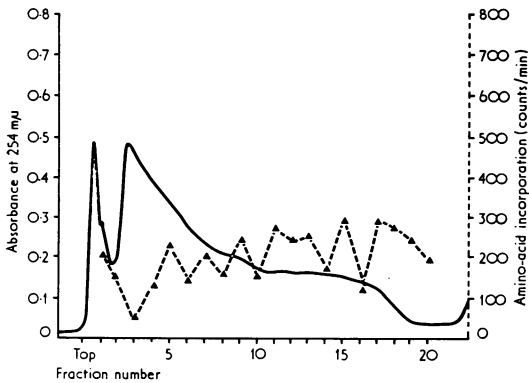


FIG. 2. Sucrose density gradient analysis of polyribosomes from muscle vastus lateralis of a 5-year-old control (no. 1). The gradient was layered with 130 μg of the major ribosomal fraction. The amount of the normal soluble enzyme used was 140 μg per tube.

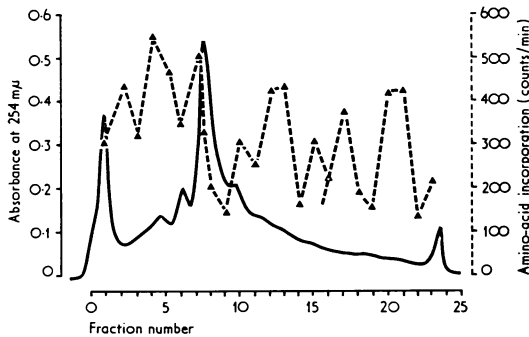


FIG. 3. Sucrose density gradient analysis of polyribosomes from muscle vastus lateralis of a 28-year-old patient with Becker MD (patient 4). The gradient was layered with 99.4 μg of the major ribosomal fraction. The amount of the dystrophic soluble enzyme used was 140 μg per tube.

In-vitro amino-acid incorporation of ribosomes. After separation of the ribosomes into 20–25 fractions by sucrose density gradient centrifugation, the incorporation of a mixture of ^{14}C -labelled amino acids (Schwartz Bioresearch 3122-08) in the presence of added soluble enzymes from the same subject was used as a measure of total protein (Ionasescu *et al*, 1971a and b). A summary of our findings is presented in Table II. In Duchenne MD the specific activity of monomeric ribosomes was normal while the average value for the amino-acid incorporation of total polyribosomes was 5.8 times higher than for our controls. The ratio of the specific activities of the polyribosomes to the monomeric ribosomes showed highly significant differences between the dystrophic and control groups. In Becker MD, the specific activity of the monomeric ribosomes was about two times higher than in

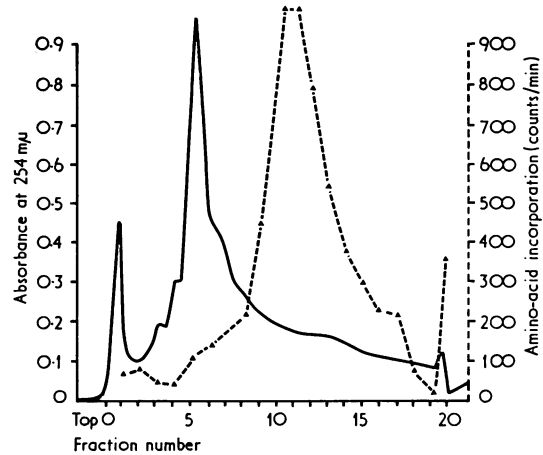


FIG. 4. Sucrose density gradient analysis of ribosomes from the deltoid muscle of a 4-year-old patient with the early stage of FSH MD (patient 1). The gradient was layered with 128 μg of the major ribosome fraction. The amount of the dystrophic soluble enzyme used was 90 μg per tube.

our controls. The protein synthesis of the total polyribosomes was significantly higher (by 4.3) than in the normal muscle. The ratio of the specific activities of the polyribosomes to the monomeric ribosomes was also significantly higher than in the control group. In FSH MD significant changes in protein synthesis were observed only in the early stage of the disease. They consisted of decrease of specific activity of monomeric ribosomes, high protein synthesis (times 3) of total polyribosomes, high ratio of the specific activities of the polyribosomes to the monomeric ribosomes. FSH MD in a late stage showed normal findings of ribosomal protein synthesis.

In-vitro synthesis of collagen. The sedimented polyribosomes (pelleted to the bottom of the 15 to 50% linear sucrose gradients) are heavy ribosomes, which normally synthesize most of the collagen and of the heavy chains of the myosin molecule. Table III gives a synopsis of our findings. In Duchenne MD, collagen synthesis was significantly increased, while noncollagen synthesis significantly decreased. Collagen production represented about 85% of the total protein synthesis in this class of ribosomes. In Becker MD, collagen synthesis showed also highly significant values, but noncollagen synthesis was within normal limits. Collagen synthesis averaged 66% of the total protein synthesis of the sedimented polyribosomes in Becker MD. In FSH MD, collagen synthesis showed normal values, while noncollagen synthesis

TABLE II
AMINO-ACID INCORPORATION OF POLYRIBOSOMES AND
MONOMERIC RIBOSOMES (cpm/ μ g of ribosomes)

MD Type	A. Monomeric Ribosomes	B. Total Polyribosomes (fractionated and sedimented)*	C. Ratio B/A
Duchenne (n=30) Controls (n=10) t p	14.7 \pm 8 18.1 \pm 3.1 -1.3 > 0.05	338.0 \pm 24 58.0 \pm 10.5 35.5 < 0.001	24.4 \pm 6.7 3.3 \pm 0.4 9.8 < 0.001
Becker (n=7) Controls (n=10) t p	35.7 \pm 7.0 18.1 \pm 3.1 7.1 < 0.01	251.1 \pm 71.3 58.0 \pm 10.5 8.5 < 0.01	7.8 \pm 3.6 3.3 \pm 0.4 4.0 < 0.01
FSH, early stage (n=4) Controls (n=5) t p	5.4 \pm 1.7 15.5 \pm 4 -4.6 < 0.01	158.0 \pm 94.8 50.0 \pm 18 2.5 < 0.05	27.2 \pm 8 3.2 \pm 1 6.7 < 0.01
FSH, late stage (n=6) Controls (n=5) t p	11.6 \pm 3 15.5 \pm 4 -1.8 > 0.05	40.2 \pm 12 50 \pm 18 -1.1 > 0.05	4.2 \pm 1.7 3.2 \pm 1 1.1 > 0.05

* Total polyribosomes include fractionated polyribosomes (light polyribosomes distributed in the sucrose density gradient) and sedimented polyribosomes (heavy polyribosomes pelleted to the bottom of the 15 to 50% sucrose density gradient).

TABLE III
PROTEIN SYNTHESIS OF SEDIMENTED MUSCLE
POLYRIBOSOMES
(cpm/ μ g of ribosomes)

MD Type	Noncollagen Protein	Collagen Protein
Duchenne (n=30) Controls (n=10) t p	129 \pm 82 244 \pm 64 -4.0 < 0.01	731 \pm 583 36 \pm 17 3.7 < 0.01
Becker (n=7) Controls (n=10) t p	219 \pm 203 244 \pm 64 -0.3 > 0.05	444 \pm 215 36 \pm 17 6.0 < 0.01
FSH (n=10) Controls (n=10) t p	160 \pm 83 244 \pm 64 -2.5 < 0.05	17.5 \pm 14 36 \pm 17 -2.6 < 0.05

was slightly lower than in the control group. The statistical significance of this decrease is borderline, and for this reason I would be tempted to consider it in the range of the normal variation. Collagen synthesis represented only about 10% of the total protein synthesis of the sedimented polyribosomes in FSH MD.

Discussion

The sedimented polyribosomes in Duchenne and Becker MD synthesize large amounts of collagen. To determine if the usually high collagen synthesis of dystrophic sedimented ribosomes is a property of ribosomal factors or of the soluble enzyme fraction from the muscle, some cross experiments were done

(Ionasescu *et al.*, 1971a). Figure 5 illustrates this kind of experiment. Normal sedimented polyribosomes and soluble enzyme fraction from Duchenne MD produce less noncollagen (contractile) protein but more collagen suggesting that dystrophic enzymes from Duchenne MD seem defective in making normal amounts of noncollagen protein. The influence of normal soluble enzymes on sedimented polyribosomes from Duchenne MD is very striking. The noncollagen protein synthesis is increased while the amount of collagen made by Duchenne sedimented polyribosomes drops dramatically to normal values. The influence of dystrophic soluble enzymes on sedimented polyribosomes belonging to carriers of Duchenne MD shows an increase in collagen synthesis with values intermediate between the normal controls and Duchenne MD, consistent with the carrier state of an inherited disease.

These results would suggest that the polyribosomes have normal function in Duchenne MD, being capable of correctly reading the messenger RNA in the presence of normal soluble enzymes. Factors present in the enzyme fraction which might account for lowering collagen synthesis in Duchenne MD include aminoacyl-tRNA synthetases, protein-chain elongation factors, tRNA's and enzyme concerned with the initiation and termination of protein synthesis.

In the early stage of Duchenne MD, the high collagen synthesis is present only in the sedimented polyribosomes. In the late stage of the disease, when

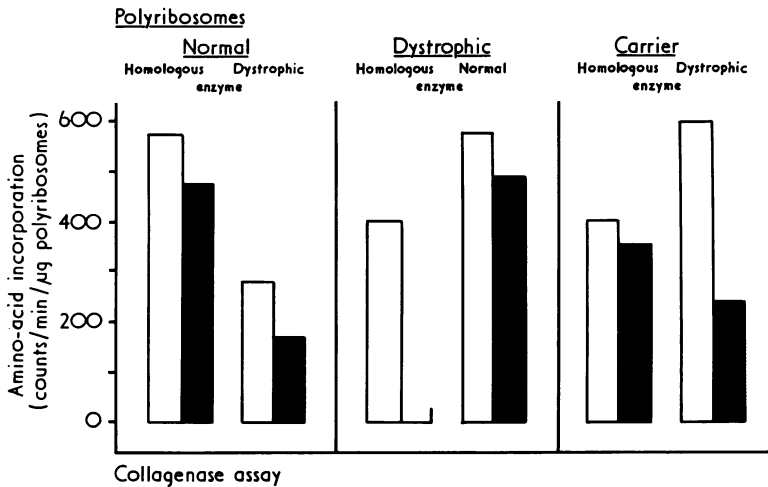


Fig. 5. Collagenase assay measuring collagen synthesis by sedimented polyribosomes complemented with soluble enzymes from patients with Duchenne MD, carriers or controls. Homologous enzyme is the soluble enzyme fraction from the same subject as the sedimented polyribosomes. The amount of the normal enzyme fraction was 140 μg ; the amount of dystrophic enzyme and of carrier enzyme was 120 μg . The open bars represent total protein synthesis and the hatched bars noncollagen protein synthesis. The amount of collagen synthesized is the difference between the total and noncollagen protein synthesized; it represents the radioactivity made acid-soluble by added bacterial collagenase.

the patient becomes wheelchair confined, all the classes of polyribosomes including the light ones synthesize mostly collagen, while the synthesis of contractile proteins is very low.

Cross experiments with sedimented polyribosomes from Becker MD and normal soluble enzymes did not show significant changes suggesting that a ribosomal factor is determining the high collagen synthesis. The latter change may be a secondary feature since it is not achieved at the expense of noncollagen protein synthesis as in Duchenne MD. The synthesis of contractile proteins shows normal values even in an advanced stage of the disease. This finding probably accounts for the benign course of this type of MD. The protein synthesis in Duchenne and Becker MD seems different enough to support the genetic evidence of Emery (1966) and Emery, Smith, and Sanger (1969) that they are non-allelic.

FSH MD has a normal protein synthesis of the sedimented polyribosomes, in contrast to Duchenne and Becker MD (see Table III). The disorder in the former condition seems to be in the light polyribosomes, which synthesize large amounts of noncollagen proteins in the early stage of the disease. The location of the peak of amino-acid incorporation would suggest a high synthesis of tropomyosin or actin (see Fig. 4). Collagen synthesis is normal in the early as well as in the late stage of the disease. Cross experiments with polyribosomes from FSH

MD and normal soluble enzymes did not give significant results. This would suggest the increase in synthesis of contractile proteins is controlled by a ribosomal factor. The specificity of alterations in ribosomal protein synthesis in FSH and Becker MD requires further studies about the amounts and types of proteins synthesized *in vitro*.

In conclusion, it seems that in the evaluation of ribosomal protein synthesis, particularly of the heavy polyribosomes, light or electron microscopy, rather than other biochemical tests, shows changes which help in distinguishing different types of muscular dystrophies. These findings do not prove or disprove whether the metabolic disorder in protein synthesis has the origin in the central nervous system or in the muscle.

We gratefully acknowledge Paul Shirk and Mrs Ruth Lewis for technical help. This study was supported in part by the Muscular Associations of America, Inc, National Institutes of Health (NS09283), USPHS Clinical Research Center Grant M01-FR-59 for patient services, and State Services for Crippled Children, Special Project Budget MR12, HEW Children's Bureau, Clinical Research in Mental Retardation and Genetic Study.

REFERENCES

- Emery, A. E. H. (1966). Genetic linkage between the loci for colour blindness and Duchenne type muscular dystrophy. *Journal of Medical Genetics*, **3**, 92-95.
- Emery, A. E. H., Smith, C. A. B., and Sanger, R. (1969). The linkage relations of the loci for benign (Becker type) X-borne muscular dystrophy, colour blindness and the Xg blood groups. *Annals of Human Genetics*, **32**, 261-269.

- Ionasescu, V., Zellweger, H., and Conway, T. W. (1971a). Ribosomal protein synthesis in Duchenne muscular dystrophy. *Archives of Biochemistry and Biophysics*, **144**, 51-58.
- Ionasescu, V., Zellweger, H., and Conway, T. W. (1971b). A new approach for carrier detection in Duchenne muscular dystrophy: Protein synthesis of muscle polyribosomes in vitro. *Neurology*, **21**, 703-709.
- Ionasescu, V., Zellweger, H., McCormick, W. F., and Conway, T. W. (1973a). Comparison of ribosomal protein synthesis in Becker and Duchenne muscular dystrophies. *Neurology*, **23**, 245-253.
- Ionasescu, V., Zellweger, H., Shirk, P., and Conway, T. W. (1972). Abnormal protein synthesis in facioscapulohumeral muscular dystrophy. *Neurology*, **22**, 1286-1292.
- Ionasescu, V., Zellweger, H., Shirk, P., and Conway, T. W. (1973b). Identification of carriers of Duchenne muscular dystrophy by muscle protein synthesis. *Neurology*, **23**, 497-502.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275.
-

Announcements

The University of Kansas Medical School in conjunction with the National Foundation—March of Dimes is sponsoring the 1975 Conference on the Clinical Delineation of Birth Defects, from 1-5 June 1975. For information write R. Neil Schimke, MD, University of Kansas Medical Center, 39th and Rainbow, Kansas City, Kansas 66103, USA.

The New York Academy of Sciences and the Institute of Society, Ethics and the Life Sciences are co-sponsors of a conference on 'Ethical and Social Questions Posed by "Engineering" the Human Genome' to be held at the Delmonico Hotel, New York City on 15 and 16 May 1975. Further information is available from: Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA.