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Dystonia Musculorum Mutation and Myosin Heavy Chain Expression in Skeletal and Cardiac Muscles

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Abstract This study evaluated the influence of dystonia musculorum (dt) mutation, characterized by spinocerebellar fibers degeneration, on cardiac and skeletal muscles: one respiratory (diaphragm, Dia), three masticatory (anterior temporalis, AT; masseter superficialis, MS; and anterior digastric, AD), one hindlimb (soleus, S), tongue (T), and one cardiac (ventricle, V). Body and muscle weight, muscle protein content, and myosin heavy chain (MHC) isoforms relative expression were then compared in dt mutant mice and in normal mice, according to sex. Male body and muscle weight was always greater than that of females, but there was no specific muscle difference in females. dt mutant mice showed a reduced whole body growth but no specific muscle atrophy, as well as a global decrease in muscle protein content that made muscles more fragile. dt mutation induced a global reduction of muscle protein concentration, whereas a general influence of sex could not be disclosed. Concerning MHC relative composition, all the muscles were fast-twitch: Dia, AT, MS, AD, S, and T expressed predominantly the fast type 2 MHC isoforms, whereas V contained only MHC α, also a fast MHC. Female muscles were slower than male muscles, except for S, which was faster. However, classification of muscles in terms of shortening velocity was very different in normal males and females. In other respects, dt mutant muscles were slower and consequently more fatigue resistant than normal, except for S, which became faster and less fatigue resistant. dt mutation exhibits then a specific effect on this continually active postural muscle. In the other muscles, global increased fatigue resistance could constitute an adaptive response to work requirements modifications linked to the muscle damage. It should be noted that a developmental MHC (neonatal) was present in female dt AD. Innervation, which influences muscle structure, is altered in dt mutant and could be another causal factor of the fast-to-slow MHC switches. It appears that dystonin, the dt gene product, is very important in maintaining the structural integrity of both cardiac and skeletal muscle and in its absence, the muscle becomes more fragile and is damaged by modified activity. J. Cell. Biochem. 74:90–98, 1999.

Key words: Friedreich's ataxia; dystonia musculorum; myosin heavy chain; mice; electrophoresis; muscle

The mouse mutant dystonia musculorum (dt) was first described by Duchen et al. [1964].This recessive hereditary neuropathy of the mouse is an animal model for human spinocerebellar ataxia. The dt mutation does not resemble that in Friedreich's ataxia, the commonest form of human degenerative ataxic disorders. Friedreich's ataxia is caused by an expanded GAA repeat, resulting in dysfunction of frataxin, a nuclear encoded mitochondrial protein [Beal, 1998]. The gene defective in dt encodes a cytoskeletal linker protein, dystonin, that forms the bridge between f-actin and intermediate filaments [Bernier and Kothary, 1998]. Disease progression is gradual in dt mice, having begun

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during embryogenesis. dt mutant mice are characterized by degeneration of two primary sensory fibers and one secondary system, the spinocerebellar projection [Janota, 1972; Sotelo and Guenet, 1988]. This latter is known to be involved in the control of limb movements [Lalonde et al., 1994]. Indeed, affected animals display loss of limb coordination and twisting of the trunk.

In humans, Friedreich's ataxia is characterized by some symptoms associated to different categories of muscles: dysarthria related to respiratory, masticatory, and tongue muscles; ataxia, muscle weakness, and loss of coordination and balance related to skeletal muscles; heart disease, heart failure, and dysrhythmias related to heart muscles.

Evidence is now available showing a correlation between muscle contractile properties and myosin heavy chain (MHC) composition [Bottinelli et al., 1996]. The MHC isoforms known in skeletal muscles are three fast types (MHC 2A, 2X, and 2B), one slow type (MHC 1 or β [Bär and Pette, 1988; Schiaffino et al., 1989], and two developmental types (MHC embryonic and neonatal [NN]) [D'Albis et al., 1989]. In the heart, two isoforms have been described: MHC β, which is common to the heart and slow skeletal muscle and MHC α cardiac [Swynghedauw, 1986]. During development, or when the working conditions are changed, marked transitions in the myosin content occur in fast- and slow-twitch muscles [Swynghedauw, 1986]. These modifications generally adapt the muscle to the new environmental requirements. Despite their similarity in primary structure, expression of different MHC isoforms is precisely regulated in a tissue- and developmental stage-specific manner [Whalen et al., 1981]. In addition, various factors, such as altered physiological stimuli [Izumo et al., 1986] and altered loading states [Baldwin, 1996], are known to cause MHC isoform switches. Thus, after a few weeks of synergistic tenotomy, fast muscles become slow, fatigue resistant, and then more adapted to endurance. Sfondrini et al. [1996] proposed that skeletal muscles could quickly adapt to functional demand, changing exclusively their fast fiber type composition. Adult skeletal muscle expresses three fast MHC isoforms, providing considerable structural and functional diversity. Indeed, MHC 2 are expressed in muscle regions used during sustained locomotion (2A and 2X) or high-power output activity (2X and 2B) [Adams et al., 1994].

To our knowledge, there is no report on the structure of the muscles related to the symptomatology, either in human ataxias or in their animal models. In order to determine dt mutation consequences on muscle, the weight, protein concentration, and MHC isoforms relative expression were studied in phonatory, cardiac, and skeletal muscles of dt mutant mice and their normal littermates. Furthermore, both male and female mice were used to determine whether the effects of mutation differed according to sex.

MATERIALS AND METHODS

Animals

A colony of mice was established at University of Nancy 1, Faculty of Medicine, from B6C3-a/a mice heterozygous for the dt gene, originally purchased from the Jackson Laboratory (Bar Harbor, ME). The dt mutation is autosomal recessive and homozygous dt mutants (dt/dt) were obtained by crossing pairs of heterozygotes (dt/+). Seven dt mutant mice (dt/dt)(4 males and 3 females) and eight normal littermate controls (+/?)(4 males and 4 females) were studied at the age of 90 days (89–91 days). Before experimentation, animals were housed in a reversed day–night cycle. Food and water were available ad libitum during the whole experiment.

Muscle Sampling and Myosin Extraction

The following muscles were removed from the mice: diaphragm (Dia, a continually active muscle even before birth), anterior temporalis (AT, elevator mandibular muscle), masseter superficialis (MS, propulsive mandibular muscle), anterior digastric (AD, depressor mandibular muscle), soleus (S, extensor of the tarsus and plantar flexor of foot, a postural and weight-bearing muscle), tongue (T), and cardiac ventricle (V). After dissection, the muscles were immediately frozen in liquid nitrogen for protein and electrophoretic analyses. After muscle wet weighing, myosin was crudely extracted in a high-ionic-strength buffer, as described by D'Albis and colleagues [1979].

Electrophoretic Analysis of MHC and Quantification

Muscle protein content was determined by using the Bradford technique with Coomasie

Protein Assay Reagent G-250 (Pierce) and a Beckman DU 640B spectrophotometer. Electrophoresis was performed according to the method of Talmadge and Roy [1993]. Mini-gels were used in the Bio-Rad Mini-protean II Dual Slab Cell. Electrophoresis took place in a refrigerated room, at a temperature of 6°C for the whole run. To separate all the heavy chains, the duration of the run was 30 h, according to Agbulut et al. [1996]. A total of 2.5 mg of protein was loaded into each well. The gels were stained with Coomassie blue R-250. The relative amounts of the different MHCs were measured using an integration densitometer Bio-Rad GS-700 and analyzed with the Molecular Analyst Program.

Statistical Analysis

After two-way analysis of variance (ANOVA), Student's *t*-test was used to establish the intergroup comparison. Differences were considered significant at $P < 0.05$. The muscle weights, protein contents, and results of electrophoretic analysis were expressed as mean ± SE.

RESULTS

Muscle weights are reported in Table I. In order to take whole body weight variations into account, the muscle weights are expressed as a percentage of the body weight shown in Table II. Male body weight (Table II) was greater than female body weight ($F = 15.7$, $P = 0.0022$). By contrast, the mutation led to a marked decrease in body weight ($F = 54.3$, $P < 0.0001$; Table II). Muscle weight was related to sex in three of the seven muscles studied (AT: $F = 14.1$, $P = 0.0032$; MS: $F = 24.6$, $P = 0.0004$; V: $F = 9.0$, $P = 0.0120$; Table I). The dt mutation reduced the weight of six muscles (Dia: $F = 7.7$, $P = 0.0181$; AT: $F = 18.9$, $P = 0.0012$; MS: $F = 69.9$, $P < 0.0001$; S: $F = 9.7$, $P = 0.0097$; T: $F = 6.5$, $P = 0.0272$; V: $F = 16.8$, $P = 0.0018$). In relative weight, two muscles were significantly affected both by dt mutation (AD: $F = 16.6$, $P = 0.0019$; T: $F = 17.9$, $P = 0.0014$) and by sex (AD: $F = 10.0$, $P = 0.0091$; T: $F = 10.8$, $P = 0.0073$). However, in Student's *t*-test, these sex differences did not reach the level of significance (Table II).

Muscle protein concentrations are presented in Table III. Protein concentration was sex dependent in five muscles (Dia: $F = 60.4$, $P < 0.0001$; MS: $F = 32.2$, $P = 0.0001$; AD: $F = 26.8$, $P = 0.0002$; S: $F = 43.1$, $P < 0.0001$; T: $F = 11.6$, $P = 0.0052$). Compared with males, in normal mice, the female muscle protein concentration

TABLE I. Muscle Weight in Normal and Dystonic Mice, According to Sex[a]

		Muscle weight (mg)						
Group	n	Diaphragm	Anterior temporalis	Masseter superficialis	Anterior digastric	Soleus	Tongue	Cardiac ventricle
Normal male	4	70.2 ± 6.6	27.5 ± 3.4	72.0 ± 3.1	3.7 ± 0.6	9.2 ± 1.4	70.2 ± 4.9	122.2 ± 5.7
Normal female	4	68.5 ± 5.1	14.7 ± 0.9**	45.5 ± 2.7**	4.9 ± 0.7	8.0 ± 1.8	72.0 ± 5.2	100.0 ± 6.2**
Dystonic male	4	61.5 ± 7.4	13.2 ± 3.1*	33.5 ± 4.5*	4.4 ± 0.3	6.0 ± 0.9	61.2 ± 4.9	91.2 ± 6.7*
Dystonic female	3	42.0 ± 5.0*	7.0 ± 0.6*	25.0 ± 3.2*	4.3 ± 0.7	2.5 ± 1.0	55.0 ± 5.1	65.3 ± 14.3

[a]Values are means ±SE.
*Different from normal of the same sex at $P < 0.05$.
**Different from male of the same phenotype at $P < 0.05$.

TABLE II. Body Weight and Muscle Weight in Percentage of Body Weight in Normal and Dystonic Mice, According to Sex[a]

			Muscle weight (% of body weight)						
Group	n	Body weight (g)	Dia-phragm	Anterior temporalis	Masseter superficialis	Anterior digastric	Soleus	Tongue	Cardiac ventricle
Normal male	4	29.1 ± 1.6	2.4 ± 0.2	0.9 ± 0.1	2.5 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	2.4 ± 0.2	4.2 ± 0.2
Normal female	4	23.6 ± 1.0**	2.9 ± 0.2	0.6 ± 0.0	1.9 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	3.0 ± 0.2	4.2 ± 0.3
Dystonic male	4	18.6 ± 1.4*	3.3 ± 0.4	0.7 ± 0.2	2.0 ± 0.2	0.2 ± 0.0*	0.3 ± 0.0	3.3 ± 0.3*	4.9 ± 0.4
Dystonic female	3	12.6 ± 1.9*,**	3.3 ± 0.4	0.6 ± 0.0	2.0 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	4.4 ± 0.4*	5.2 ± 1.1

[a]Values are means ±SE.
*Different from normal of the same sex at $P < 0.05$.
**Different from male of the same phenotype at $P < 0.05$.

TABLE III. Protein Concentration (μg per mg muscle) in Normal and Dystonic Mice Muscles, According to Sex

	Protein concentration (μg per mg muscle)						
Group	Diaphragm	Anterior temporalis	Masseter superficialis	Anterior digastric	Soleus	Tongue	Cardiac ventricle
Normal male	22.6 ± 0.4	16.7 ± 0.1	17.9 ± 0.4	9.6 ± 0.1	15.4 ± 0.0	17.8 ± 0.1	22.3 ± 0.3
Normal female	18.4 ± 0.3**	17.7 ± 0.6	23.5 ± 0.4**	7.9 ± 0.3**	16.0 ± 0.4	15.9 ± 0.2**	21.4 ± 0.5
Dystonic male	19.2 ± 0.3*	11.0 ± 0.4*	15.5 ± 0.5*	7.5 ± 0.3*	11.7 ± 0.1*	13.2 ± 0.1*	14.5 ± 0.1*
Dystonic female	18.6 ± 0.1	11.2 ± 0.1*	14.4 ± 0.2*	11.3 ± 0.2*,**	14.0 ± 0.1*,**	12.7 ± 0.6*	17.6 ± 1.0*,**

Values are means ±SE.
*Different from normal of the same sex at $P < 0.05$.
**Different from male of the same phenotype at $P < 0.05$.

was significantly lower in Dia, AD, and T, and higher in MS (Table III). In mutant mice, the female muscle protein concentration was significantly higher in AD, S, and V versus that in male muscles. By contrast, the dt mutation altered the protein concentration of all studied muscles (Dia: F = 28.1, $P = 0.0002$; AT: F = 274.6, $P < 0.0001$; MS: F = 204.3, $P < 0.0001$; AD: F = 9.9, $P = 0.0085$; S: F = 179.0, $P < 0.0001$; T: F = 117.8, $P < 0.0001$; V: F = 106.2, $P < 0.0001$). In males, protein concentration was significantly decreased by mutation in all muscles, whereas in females, it was decreased only in five muscles (AT, MS, S, T, and V), increased in AD and unchanged in Dia. Furthermore, there was a significant interaction between sex and phenotype on protein concentration in five muscles (Dia: F = 36.1, $P < 0.0001$; MS: F = 69.2, $P < 0.0001$; AD: F = 167.4, $P < 0.0001$; S: F = 11.6, $P = 0.0052$; V: F = 13.4, $P = 0.0033$). Consequently, the modifications of protein concentration cannot be explained by the sex or the phenotype factors alone, but by a combination of these two factors.

The electrophoretic data of the four pooled groups are illustrated in Figure 1 for each muscle type, and the densitometric analysis is presented in Table IV. The resulting bands are, in order of increasing electrophoretic mobility, adult fast 2A, adult fast 2X, neonatal (NN), adult fast 2B, and slow adult 1 types (Fig. 1). The proportions of MHC isoforms were expressed as a relative percentage of the total amount of MHC present in the muscles studied (Table IV).

In the three normal masticatory muscles (AT, MS, AD), the three adult fast MHC isoforms could be observed, MHC 2A, 2X, and 2B, except for MS of normal males in which MHC 2A was not expressed (Table IV). The Dia and S expressed the three adult fast MHC isoforms, MHC 2A, 2X, and 2B and the slow type, MHC 1. The T only expressed MHC 2X and 2B. Finally, the V muscle MHC composition (only MHC α) is not presented in Figure 1 and Table IV, because it was unaffected by sex and phenotype.

Sex affected the MHC relative expression both in normal and in mutant mice muscles. In the first place, sex modified significantly the MHC relative expression in all the normal muscles studied (Table IV). The female Dia presented more MHC 1 and less MHC 2B than that of males. However, whatever sex, the Dia of normal mice expressed mainly MHC 2A and 2X. The male AT expressed predominantly MHC 2B (77%), whereas the female AT expressed mainly MHC 2A (43%) and 2X (48%). The male and female MS and AD expressed mainly MHC 2X (>60%). Nevertheless, in these two muscles, the second major isoform was MHC 2B (>30%) in male, and MHC 2A (>15%) in female. The male MS presented no MHC 2A at all. The female S profile was composed principally of the three fast MHC in equivalent proportions (approximately 30%), while the male S expressed mainly MHC 2B (48%). In male T muscle, MHC 2B was the predominant isoform (71%), whereas in the female T, almost equal amounts of MHC 2X and 2B were present. In the second place, sex modified significantly the MHC relative expression in all the mutant muscles studied (Table IV). Whereas the female mutant Dia expressed equal amounts of MHC 2A and 2X, the male mutant Dia expressed mainly MHC 2A. The male AT expressed predominantly MHC 2B, whereas the female AT expressed mainly MHC 2A. In the MS muscle of male dystonic mice, MHC 2X was the predominant isoform (70%), when MHC 2A and 2X were equally expressed in females. Both male and

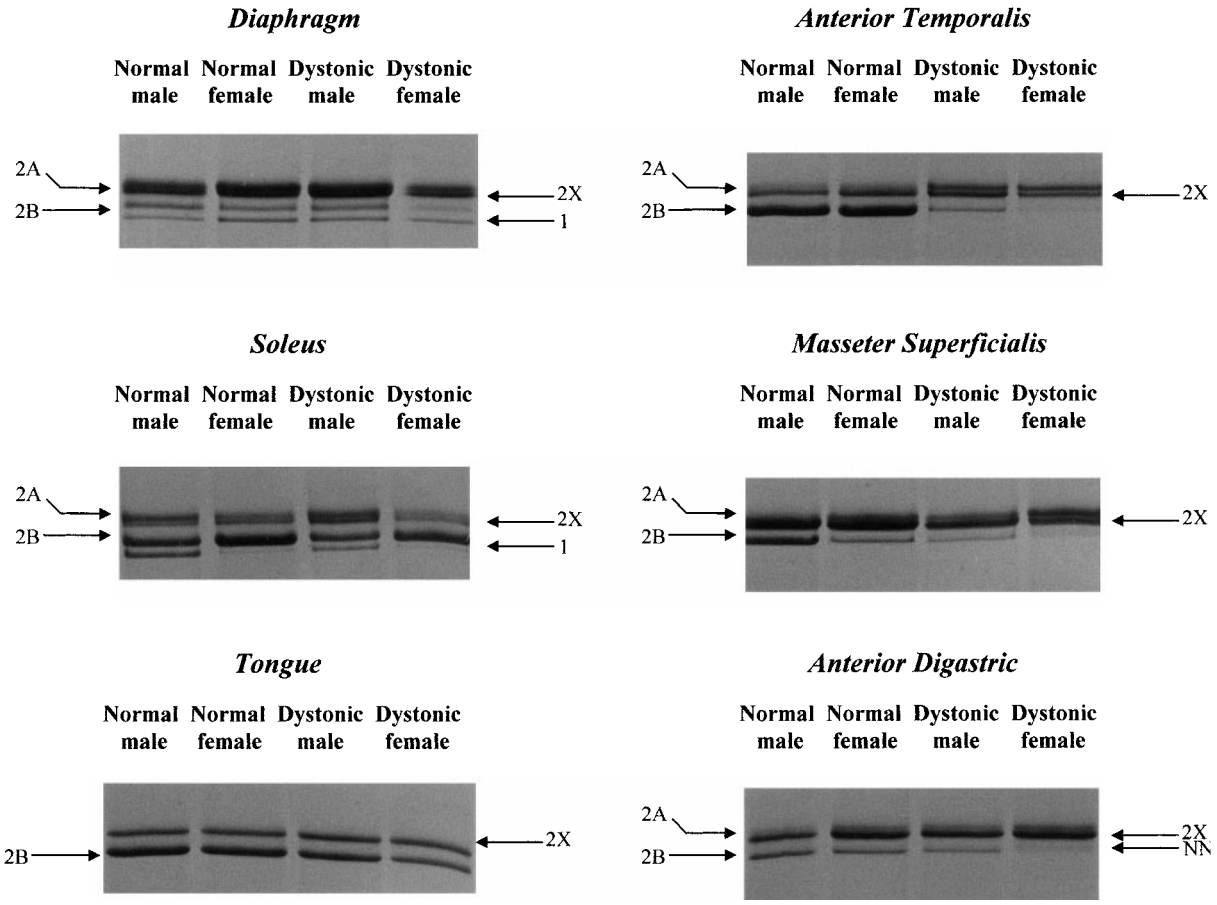

Fig. 1. Myosin heavy chain (MHC) in six muscles of adult normal and dystonic mice according to sex. Order of increasing electrophoretic mobility: adult fast 2A, adult fast 2X, neonatal (NN), adult fast 2B, and slow adult 1 types.

female dystonic AD expressed principally MHC 2X (>72%); nevertheless, female muscle expressed more MHC 2A and less MHC 2B than did male muscle. The major isoform in male and female dystonic S was MHC 2B (>76%), but the female S showed higher levels of MHC 2X and 2B and lower levels of MHC 2A. In the male T, the predominant isoform was MHC 2B, whereas in the female it was MHC 2X.

dt mutation affected the MHC relative expression in both male and female mouse muscles. In Dia, the mutation led to a reduced expression of MHC 2B and an overexpression of MHC 1, in both males and females. Furthermore, in male Dia, the dystonic muscle presented a higher level of MHC 2A and a lower level of MHC 2X than normal muscle. The dystonic AT muscle presented a lower expression of MHC 2B both in male and in female versus normal, an increase of MHC 2X in male, as compared with a decrease in female, and finally an overexpression of MHC 2A only in females. In MS, the mutation led to an increase in MHC 2A to the detriment of MHC 2X and 2B in females and only of 2B in males. In AD, the mutation led to a decrease in MHC 2B in aid of 2A and 2X in males and of 2A and NN in females. It should be noted that MHC NN was only detected in dystonic females AD. The mutant S muscle presented a lower level of MHC 2A and 1 and a higher level of MHC 2B both in males and females, whereas MHC 2X was increased in males and decreased in females. The mutant T was affected in the same way in male and female, i.e., decreased in MHC 2B and increased in 2X.

DISCUSSION

In normal and mutant mice, body weight was always greater in males than in females. Furthermore, the mutation was accompanied by a decrease in body weight. Muscle weight was

TABLE IV. Densitometric Analysis of Myosin Heavy Chain (MHC) Composition in Normal and Dystonic Mice According to Sex[a]

Group	MHC, %				
	2A	2X	2B	1	NN
			Diaphragm		
Normal male	42.4 ± 1.0	43.1 ± 0.7	12.0 ± 0.2	2.5 ± 0.1	ND
Normal female	47.9 ± 2.3	41.3 ± 2.1	6.7 ± 0.1**	4.1 ± 0.2**	ND
Dystonic male	54.9 ± 2.4*	32.8 ± 1.9*	6.2 ± 0.3*	6.1 ± 0.3*	ND
Dystonic female	47.9 ± 1.8	44.1 ± 1.3**	2.7 ± 0.2*,**	5.3 ± 0.4*	ND
			Anterior temporalis		
Normal male	4.6 ± 0.4	18.0 ± 0.3	77.4 ± 0.3	ND	ND
Normal female	43.2 ± 0.8**	48.3 ± 0.6**	8.5 ± 0.3**	ND	ND
Dystonic male	6.0 ± 0.7	23.3 ± 0.8*	70.7 ± 0.5*	ND	ND
Dystonic female	60.3 ± 1.0*,**	38.9 ± 1.1*,**	0.8 ± 0.3*,**	ND	ND
			Masseter superficialis		
Normal male	ND	66.2 ± 0.2	33.8 ± 0.2	ND	ND
Normal female	23.4 ± 1.2**	71.9 ± 1.2**	4.7 ± 0.2**	ND	ND
Dystonic male	24.0 ± 1.6*	69.0 ± 1.6	7.0 ± 0.1*	ND	ND
Dystonic female	51.7 ± 1.3*,**	47.7 ± 1.3*,**	0.6 ± 0.2*,**	ND	ND
			Anterior digastric		
Normal male	6.8 ± 0.8	62.3 ± 0.7	30.9 ± 0.4	ND	ND
Normal female	15.4 ± 1.1**	76.2 ± 1.3**	8.4 ± 0.2**	ND	ND
Dystonic male	14.4 ± 1.1*	72.2 ± 1.3*	13.4 ± 0.3*	ND	ND
Dystonic female	24.1 ± 0.8*,**	74.6 ± 0.9	0.3 ± 0.1*,**	ND	1.0 ± 0.1*,**
			Soleus		
Normal male	23.8 ± 0.9	8.7 ± 0.3	47.5 ± 0.3	20.0 ± 0.8	ND
Normal female	30.5 ± 0.8**	25.7 ± 0.4**	36.9 ± 0.3**	6.9 ± 0.2**	ND
Dystonic male	11.2 ± 0.5*	12.4 ± 0.1*	76.1 ± 0.4*	0.3 ± 0.1*	ND
Dystonic female	7.3 ± 0.4*,**	13.3 ± 0.2*,**	78.7 ± 0.2*,**	0.7 ± 0.2*	ND
			Tongue		
Normal male	ND	29.2 ± 0.5	70.8 ± 0.5	ND	ND
Normal female	ND	43.2 ± 0.2**	56.8 ± 0.2**	ND	ND
Dystonic male	ND	32.9 ± 0.2*	67.1 ± 0.2*	ND	ND
Dystonic female	ND	55.3 ± 0.2*,**	44.7 ± 0.2*,**	ND	ND

ND, not detected (<1%).
[a]Values are percentages of total MHC ±SE.
*Different from normal of the same sex at $P < 0.05$.
**Different from male of the same phenotype at $P < 0.05$.

related to sex in AT, MS, and V and to phenotype in Dia, AT, MS, S, T, and V. More precisely, male muscles were heavier than female muscles in mutant as well as in normal mice, and the mutant muscles were lighter than normal muscles. Nevertheless, because a linear relation has been shown between skeletal muscle and body weight [Uchiyama et al., 1994], these variations can be attributed principally to body weight modifications. Indeed, in relative weight, these reductions were not observable. On the contrary, the relative weight of two muscles, AD and T, increased significantly in mutant mice. It appears that whereas females are always globally smaller than males in both normal and dystonic mice, the dt mutation induces a reduction in whole body growth but no specific muscle atrophy.

Protein concentration was modified considerably by sex and dt mutation. In normal mice,

the protein concentration of muscle was lower in females in Dia, ADs and T, comparable in AT, S, and V, and higher in MS. In mutant mice, females showed a higher protein concentration in AD, S, and V, but comparable concentrations in other muscles. Therefore, a general influence of sex on muscle protein concentration cannot be disclosed from these results. dt mutation induced a global reduction of muscle protein content, except for female Dia, in which it was comparable and for female AD in which it was increased.

With respect to maximum velocity of shortening, the type 2B fiber is thought to have the highest velocity, followed by 2X > 2A > 1 [Bottinelli et al., 1991]. These investigators evaluated the maximum velocity of shortening for the different fiber types, in rat skeletal muscles, in muscle lengths per second: 0.639 ± 0.038 (mean ± SE) for type 1, 1.396 ± 0.084 for type 2A, 1.451 ± 0.066 for type 2X, and 1.800 ± 0.109 for type 2B. Furthermore, according to Nelson and Thompson [1994], neonatal muscles contain motor units of differing contractile properties and myosin composition. These authors classified the neonatal single motor units depending on their unloaded shortening velocity. The slowest motor units contained MHC 1, intermediate motor units contained both MHC E, NN and/or 2A, and finally, the fastest motor units contained MHC NN and/or 2A. The velocity of motor units containing adult fast myosins would then be slowed by the presence of either or both developmental MHCs. Consequently, our results show that, except for V, the muscles studied (Dia, AT, MS, AD, S, and T), which express predominantly the type 2 MHC isoforms, are fast-twitch muscles. The V contains only MHC α, which is a fast MHC as well [Hughes et al., 1993]. Therefore, V is also a fast-twitch muscle. This result is in accordance with previous observations [Agbulut et al., 1996]. These workers showed that mouse newborn heart contained MHC α and β, but that MHC β was only present during development and disappeared soon after birth.

In hybrid fibers, shortening velocity is nonlinearly related to MHC isoform composition. However, in normal male mice, the AT and T muscles, containing mainly type 2B, are faster than the MS and AD muscle, containing mainly 2X, which in turn are faster than the Dia and S muscles, in which MHC 2A and 1 represent 40%. By contrast, in female normal mice, T is the fastest muscle, with 57% of MHC 2B, followed by S (MHC 2B = 37%), which is faster than AD, MS (expressing principally MHC 2X), and AT (MHC 2A = 40% and 2X = 50% approximately) and finally Dia, the slowest muscle in which MHC 2A and 2X represent 50% and 40%, respectively. The classification of muscles in terms of shortening velocity is, then, very different in normal male and female. For instance, AT is the fastest muscle studied in normal male and is next to last in velocity in female. To our knowledge, this AT sexual dimorphism has never been reported in mice. However, on the basis of histochemical classification, Lyons et al. [1986] showed a similar sexual dimorphism in guinea pig AT. Globally, female muscles are slower than male muscles, except for the S muscle, which is faster in females. Therefore, it appears that sexual dimorphism is muscle-type specific.

On the other hand, in mutant male mice, the S, AT, and T muscles, which contain mainly type 2B (>67%), are faster than AD and MS muscles, which contain mainly 2X (>71%), which in turn are faster than the Dia muscle, in which MHC 2A and 1 represent 61%. On the contrary, in female mutant mice, S is the fastest muscle, with 79% of MHC 2B, followed by T (MHC 2B = 45% and 2X = 55%), which is faster than AD (expressing principally MHC 2X), MS (expressing almost equal levels of MHC 2A and 2X), and AT (expressing principally MHC 2A) and finally Dia, the slowest muscle in which MHC 2A and 1 represent 48% and 5%, respectively. As in normal mice, the classification of muscles in terms of shortening velocity is then very different in mutant males and female. In particular, AT is one of the fastest muscles studied in mutant male and is next to last in velocity in female. Globally, once again as in normal mice, dystonic female muscles are slower than male muscles, except for the S muscle, which is faster in females.

Apart from V, dt mutation affected significantly MHC relative expression in the six muscles studied, in male and female mice. First, in male muscles, except for S, mutation led to a significant decrease in fast MHC 2B and an increase in slower MHCs: MHC 2A and 2X in AD, MHC 2X in AT and T, MHC 2A in MS, MHC 2A and 1 in Dia. These five muscles were then slower in mutant males than in normal males. On the contrary, the S muscle of mutant males, which showed higher levels of MHC 2X and 2B

and lower levels of MHC 2A and 1, became faster than that of normal males. Second, in female muscles, MHC 2B also decreased in all muscles except for S, but it was accompanied by an increase in MHC 1 in Dia, a decrease in MHC 2X and an increase in MHC 2A in AT and MS, an increase in MHC 2A in AD and an increase in MHC 2X in T. These five muscles, such as those of males, were then slower in mutant females than in normal females. As in males, modifications induced by mutation in S were different from the other muscles. Contrary to male, the female mutant S showed more MHC 2B and less MHC 1, 2A and 2X, but, as in males, it became faster than normal S. Therefore, the S muscle appears to be affected in a peculiar manner by sex and phenotype. It altered in the opposite direction with regard to the other muscles: S was faster in females than in males and faster in mutants than in normal mice, whereas Dia, AT, MS, AD, and T were slower in these two cases. Sieck et al. [1996] have classified the muscular motor units physiologically as slow-twitch fatigue resistant (MHC 1), fast-twitch fatigue resistant (MHC 2A), fast twitch fatigue-intermediate (MHC 2X alone or 2X and 2A), and fast twitch fatiguable (MHC 2X and 2B or 2B alone). As the dt mutation decreased the relative proportion of the fastest MHCs and increased that of the slowest MHCs in all muscles except S and V, this transition was accompanied by an increase in muscle fatigue resistance.

In summary, the dt mutant mice showed a reduced whole body growth, but no specific muscle atrophy. Furthermore, the absence of dystonin was accompanied by a global decrease in muscle protein content, which makes muscles more fragile. The remaining muscle fibers then have to work harder. The increased fatigue resistance could therefore constitute an adaptive response to these work requirements. Altered loading states are known to cause MHC isoform switches [Baldwin, 1996]. The adaptive process in response to overloading results in a net transformation in contractile protein phenotype favors the predominant expression of slower MHC isoforms. Actually, except for S, the dt mutant muscles are slower than normal muscles. Altered loading states could therefore constitute one of the factors at the origin of these MHC isoform switches. S, a continually active postural muscle, is faster and consequently less fatigue resistant in dt mice. This lower fatiguability resistance could be linked to the dt mutant hypoactivity described by Lalonde et al. [1994]. In Friedreich's ataxia patients, Beauchamp et al. [1995] described a fairly regular and statistically significant pattern of slowly progressive and symmetrical loss of strength affecting mainly the lower limbs. According to these investigators, weakness did not appear to be the primary cause of loss of ambulation. Our results suggest that loss of ambulation could be attributable, in part, to the reduced fatigue resistance of lower limb postural muscles. Innervation is also a factor influencing muscle structure. In fact, it has been shown that denervation induced selective progressive atrophy of most fast fibers and hypertrophy of many slow fibers in fast-twitch (gastrocnemius) and slow-twitch (S) muscles [D'Albis et al., 1995], as well as a decrease in the number of type 2 fibers in S [Narusawa, 1985]. dt mutant mice now exhibit motor control deficits [Strazielle et al., 1998] and primary sensory fibers degeneration [Sotelo and Guenet, 1988]. The slower MHC profiles observed in six muscles of dystonic mice could then be explained by these deficits. By contrast, the modifications observed in masticatory and respiratory muscles of dt mutant mice could contribute to identify the origin of Friedreich's ataxia dysarthria. Cardiac failure is the most common cause of death in Friedreich's ataxia patients. Casazza et al. [1990] reported a ventricular hypertrophy in these patients, associated to a massive interstitial fibrosis with cellular degeneration. The V of dt mutant mice also showed an hypertrophy (not statistically significant, however), accompanied by decreased in protein concentration. According to our results, it dystonin appears to be essential in maintaining the structural integrity of both cardiac and skeletal muscle. In its absence, the muscle is less concentrated in proteins, and its contractile properties are modified according to muscle type. Further experiments, especially ontogenic studies, are now needed to define the respective roles of sensory and motor denervation and altered loading in development and structure of dt mutant mouse muscles and to contribute to a better understanding of human ataxias.

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REFERENCES

Adams GR, Haddad F, Baldwin KM. 1994. Interaction of chronic creatine depletion and muscle unloading: effects on postural locomotor muscles. J Appl Physiol 77:1198–1205.

Agbulut O, Li Z, Mouly V, Butler-Browne GS. 1996. Analysis of skeletal and cardiac muscle from desmin knock-out and normal mice by high resolution separation of myosin heavy-chain isoforms. Biol Cell 88:131–135.

Baldwin KM. 1996. Effects of altered loading states on muscle plasticity: what have we learned from rodents? Med Sci Sports Exerc 28:101–106.

Bär A, Pette D. 1988. Three fast myosin heavy chains in adult rat skeletal muscle. FEBS Lett 235:153–155.

Beal MF. 1998. Mitochondrial dysfunction in neurodegenerative diseases. Biochim Biophys Acta 1366:211–223.

Beauchamp M, Labelle H, Duhaime M, Joncas J. 1995. Natural history of muscle weakness in Friedreich's ataxia and its relation to loss of ambulation. Clin Orthop 311: 270–275.

Bernier G, Kothary R. 1998. Prenatal onset of axonopathy in dystonia musculorum mice. Dev Genet 22:160–168.

Bottinelli R, Schiaffino S, Regianni C. 1991. Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle. J Physiol (Lond) 437:655–672.

Bottinelli R, Canepari M, Pellegrino MA, Regianni C. 1996. Force-velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence. J Physiol (Lond) 495:573–586.

Casazza F, Ferrari F, Piccone U, Maggiolini S, Capozi A, Morpurgo M. 1990. Progression of cardiopathology in Friedreich ataxia: clinico-instrumental study. Cardiologia 35:423–431.

D'Albis A, Pantaloni C, Bechet JJ. 1979. An electrophoretic study of native myosin isozymes and of their subunit content. Eur J Biochem 99:261–272.

D'Albis A, Couteaux R, Janmot C, Roulet A. 1989. Specific programs of myosin expression in the postnatal development of rat muscles. Eur J Biochem 183:583–590.

D'Albis A, Couteaux R, Goubel F, Janmot C, Mira JC. 1995. Response to denervation of rabbit soleus and gastrocnemius muscles. Time-course study of postnatal changes in myosin isoforms, fiber types, and contractile properties. Biol Cell 85:9–20.

Duchen LW, Strich ST, Falconer DS. 1964. Clinical and pathological studies of an hereditary neuropathy in mice (dystonia musculorum). Brain 91:803–818.

Hughes SM, Cho M, Karsch-Mizrachi I, Travis M, Silberstein L, Leinwand LA, Blau HM. 1993. Three slow myosin heavy chains sequentially expressed in developing mammalian skeletal muscle. Dev Biol 158:183–199.

Izumo S, Nadal-Ginard B, Mahdavi V. 1986. All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. Science 231:597–600.

Janota I. 1972. Ultrastructural studies of an hereditary sensory neuropathy in mice (dystonia musculorum). Brain 95:529–536.

Lalonde R, Joyal CC, Botez MI. 1994. Exploration and motor coordination in dystonia musculorum mutant mice. Physiol Behav 56:277–280.

Lyons GE, Kelly AM, Rubinstein NA. 1986. Testosterone-induced changes in contractile protein isoforms in the sexually dimorphic temporalis muscle of the guinea pig. J Biol Chem 261:13278–13284.

Narusawa M. 1985. Change in fiber type in partially-denervated soleus muscle of the rat Tokai. J Exp Clin Med 10:499–507.

Nelson AG, Thompson WJ. 1994. Contractile properties and myosin phenotype of single motor units from neonatal rats. Am J Physiol 266:C919–924

Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K, Lomo T. 1989. Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. J Muscle Res Cell Motil 10:197–205.

Sfondrini G, Reggiani C, Gandini P, Bovenzi R, Pellegrino MA. 1996. Adaptations of masticatory muscles to a hyperpropulsive appliance in the rat. Am J Orthod Dentofac Orthop 110:612–617.

Sieck GC, Founier M, Prakash YS, Blanco CE. 1996. Myosin phenotype and SDH enzyme variability among motor unit fibers. J Appl Physiol 80:2179–2189.

Sotelo C, Guenet JL. 1988. Pathologic changes in the CNS of dystonia musculorum mutant mouse: an animal model for human spinocerebellar ataxia. Neuroscience 27:403–424.

Strazielle C, Lalonde R, Amdiss F, Botez MI, Hebert C, Reader TA. 1998. Distribution of dopamine transporters in basal ganglia of cerebellar ataxic mice by [125I]RTI-121 quantitative autoradiography. Neurochem Int 32: 61–68.

Swynghedauw B. 1986. Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. Physiol Rev 66:710–771.

Talmadge RJ, Roy RR. 1993. Electrophoretic separation of skeletal muscle myosin heavy chain. J Appl Physiol 75: 2337–2340.

Uchiyama S, Tamaki T, Nakano S. 1994. A study of absolute and relative growth on rat skeletal muscle. Nippon Seirigaku Zasshi 56:7–16.

Whalen RG, Sell SM, Butler-Browne GS, Schwartz K, Bouveret P, Pinset-Härström I. 1981. Three myosin heavy-chain isozymes appear sequentially in rat muscle development. Nature 292:805–809.