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Influence of the Lurcher Mutation on Myosin Heavy Chain Expression in Skeletal and Cardiac Muscles

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Abstract This study evaluated the influence of Lurcher mutation, characterized by degeneration of cerebellar Purkinje cells, granule cells, and inferior olive neurons, on cardiac and skeletal muscles: one respiratory (diaphragm, Dia), three masticatory (anterior temporalis, AT; masseter superficialis, MS and anterior digastric, AD), one hind limb (soleus, S), entire tongue (T), and one cardiac (ventricle, V) muscles. Body and muscle weight, muscle protein content, and myosin heavy chain (MHC) isoforms relative expression were then compared in Lurcher mutant mice vs. normal, according to sex. Male body weight was always greater than female one, but there was no specific muscle difference in females, except for T relative weight which was greater in normal females. Muscle protein concentration was greater in normal males except for AD and T in which it was lower. Lurcher mutant mice showed a reduced whole body growth but no specific muscle atrophy (except in male AT), and a global decrease in muscle protein content which made muscles more fragile (except in female Dia and male T, in which it was greater). Lurcher 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 predominantly expressed the fast type 2 MHC isoforms, except female S, whereas V contained only MHC α, also a fast MHC. Female muscles were slower than male ones and classification of muscles in terms of shortening velocity was comparable in normal male and female. In other respects, male Lurcher mutant muscles were slower and consequently more fatigue resistant than normal, except T which became faster and less fatigue resistant. On the contrary, in female mutants, only the Dia was slower than normal one, MS and AD were comparable to normal ones and finally, AT, S, and T were faster than normal ones. It should be noted that a developmental MHC (neonatal) was present in Lurcher AD. Motor control, which influences muscle structure, is altered in Lurcher mutant and could be one of the causal factor of the fast-to-slow MHC switches observed in some mutant muscles. It seems therefore that cerebellar Purkinje cells, granule cells, and inferior olive neurons are very important in maintaining the structural integrity of both cardiac and skeletal muscle, and their degeneration is accompanied by important muscles modifications. J. Cell. Biochem. Suppl. 36:222–231, 2001.

Key words: Lurcher; OPCA; myosin heavy chain; mice; electrophoresis; muscle

The cerebellar mutant mouse Lurcher (Lc) was first described by Phillips [1960]. It is characterized by a genetically determined degeneration of cerebellar Purkinje cells, granule cells, and inferior olive neurons [Caddy and Biscoe, 1979; Wetts and Herrup, 1982a, b]. It is a suggested animal model for human olivopontocerebellar atrophy (OPCA). OPCA refers to a group of ataxias characterized by progressive neurological degeneration affecting the cerebellum, the pons, and the inferior olives. The symptoms of OPCA differ from person to person. Most patients experience difficulty with balance and coordination of the legs and arms (ataxia) and slurred speech (dysarthria) [Gilman and Quinn, 1996; Wenning et al., 1996]. Other symptoms may include muscle spasms or weakness and stiffness of the muscles, numbness or tingling of the hands or feet, tremor (shaking) of the hand or arm, reduction or slowness of movements, loss of thinking and/or memory skills, difficulty in controlling the

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bladder or bowels, and feeling faint when standing up. Generally, symptoms of OPCA begin in mid-adult life and progress slowly over the course of many years. There is no cure for OPCA. The disorder is slowly progressive with death usually occurring ~20 years after onset. There has been great progress recently since the genes for several of the hereditary forms of OPCA have been found [Rosenberg, 1995].

Lc is a gain-of-function mutation in the delta2 glutamate receptor (GRID2) that results in the cell-autonomous death of cerebellar Purkinje cells in heterozygous Lurcher (+/Lc) mice [Zuo et al., 1997]. This in turn triggers the massive loss of afferent granule cells during the first few postnatal weeks. Evidence suggests that the death of Purkinje cells as a direct consequence of GRID2 (Lc) activation and the secondary death of granule cells because of target deprivation occur by apoptosis. Motor coordination deficits have been described in Lc mutants, such as increased latencies to reach a diagonal bar from a horizontal position and an increased number of falls from a vertical grid [Lalonde et al., 1992]. Indeed, affected animals display loss of limb coordination and twisting of the trunk.

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 (E) 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 [Jamali et al., 2000]. 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 like altered physiological stimuli and altered loading states are known to cause MHC isoform switches [Baldwin, 1996; Di Maso et al., 2000]. 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. On the other hand, chronic reductions in neuromuscular activity result generally in increased expression of fast MHC isoforms at the protein and/or mRNA levels in slow and fast muscles [for review see Talmadge, 2000]. The specific fast MHC isoforms that are induced are usually the MHC 2X isoform in slow muscle and the MHC 2B isoform in fast muscle, the degree and rate of adaptation depending upon the animal species and the model studied. 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, neither in OPCA patients nor in their animal model. In order to determine Lc mutation consequences on muscle, the weight, protein concentration and MHC isoforms relative expression were studied in phonatory, cardiac, and skeletal muscles of Lc mutant mice and their normal littermates. Furthermore, both male and female mice were used to determine if the effects of mutation differed according to sex.

MATERIALS AND METHODS

Animals

A colony of B6CBACA/A$^{w\text{-}J}$ mice, heterozygous for the Lc gene (+/Lc), was established in our laboratory from stock originally obtained at Jackson Laboratory (Bar Harbor, ME). The Lc mutation is semi-dominant. Ten Lc mutant mice (+/Lc), five males and five females, and seven normal littermate controls (+/+), five males and two females, were studied at the age of 90 days. Prior to experimentation, animals were housed in 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 mus-

cle), Anterior Digastric (AD, depressor mandibular muscle), Soleus (S, extensor of the tarsus, a postural and weight-bearing muscle), entire Tongue (T) and, cardiac Ventricle (V). After dissection, the muscles were immediately frozen in liquid nitrogen for protein and electrophoretic analyses. Myosin was crudely extracted in a high ionic strength buffer, as described by d'Albis et al. [1979].

Electrophoretic Analysis of MHC and Quantification

Muscle protein content was determined by using the Bradford technique with Coomassie Protein Assay Reagent G-250 (Pierce) and a Beckman DU 640B spectrophotometer.

Electrophoresis was performed according to the method of Talmadge and Roy [1993]. Minigels 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]. 2.5 μg 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

The body and muscle weights are reported in Table I. In order to take whole body weight variations into account, the muscle weights are expressed in percent of the body weight in Table II. Male body weight (Table I) was greater than female one ($F = 34.7$, $P < 0.0001$). On the other hand, the mutation led to a marked decrease in body weight ($F = 66.8$, $P < 0.0001$). Muscle weight was related to sex only in one out of the seven muscles studied (V: $F = 9.1$, $P = 0.0098$). The Lc mutation reduced the weight of six muscles (Dia: $F = 14.3$, $P = 0.0023$; AT: $F = 42.2$, $P < 0.0001$; MS: $F = 25.8$, $P = 0.0002$; AD: $F = 13.9$, $P = 0.0025$; S: $F = 12.5$, $P = 0.0036$; V: $F = 11.4$, $P = 0.0049$). In relative weight, two muscles were significantly affected by Lc mutation (AT: $F = 14.5$, $P = 0.0022$; S: $F = 4.8$, $P = 0.0466$) and one by sex (T: $F = 7.3$, $P = 0.0183$).

Muscle protein concentrations are presented in Table III. Protein concentration was sex dependant in all the muscles studied (Dia: $F = 2786.6$, $P < 0.0001$; AT: $F = 20000.8$, $P < 0.0001$; MS: $F = 9322.7$, $P < 0.0001$; AD: $F = 41479.7$, $P < 0.0001$; S: $F = 3336.9$, $P < 0.0001$; T: $F = 16477.9$, $P < 0.0001$; V: $F = 4312.9$, $P < 0.0001$). Compared to male, in normal mice, the female muscle protein concentration was significantly lower in Dia, AT, MS, S, and V, and higher in AD and T (Table III). In mutant mice, the female muscle protein concentration was significantly higher in Dia, S, and T vs. male, and lower in the other muscles studied. On the other hand, the Lc mutation altered the protein concentration of all studied muscles (Dia: $F = 1665.7$, $P < 0.0001$; AT: $F = 18949.9$, $P < 0.0001$; MS: $F = 14190.3$, $P < 0.0001$; AD: $F = 92854.3$, $P < 0.0001$; S: $F = 4781.0$, $P < 0.0001$; T: $F = 1936.8$, $P < 0.0001$; V: $F = 2117.1$, $P < 0.0001$). Whatever sex, protein concentration was significantly decreased by mutation in all muscles, except in male T and in female Dia in which it was increased. Furthermore, there was a significant interaction between sex and phenotype on protein concentration in all the muscles studied (Dia: $F = 17940.7$, $P < 0.0001$; AT: $F = 1107.0$, $P < 0.0001$; MS: $F = 2541.6$, $P < 0.0001$; AD: $F = 51080.5$, $P < 0.0001$; S: $F = 3791.0$, $P < 0.0001$; T: $F = 9720.9$, $P < 0.0001$; V: $F = 122.8$, $P < 0.0001$).

The electrophoretic data are illustrated in Figure 1 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, 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, and 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,

TABLE I. Muscle Weight in Normal and Lurcher Mice According to Sex

		Muscle weight (mg)						
Group	n	Diaphragm	Anterior Temporalis	Masseter Superficialis	Anterior Digastric	Soleus	Tongue	Cardiac Ventricle
Normal male	5	97.4 ± 8.4	24.6 ± 1.4	59.6 ± 4.8	14.6 ± 1.4	7.8 ± 1.0	62.8 ± 2.9	116.4 ± 7.9
Normal female	2	95.0 ± 1.0	18.3 ± 3.2	55.3 ± 1.1	14.0 ± 1.0	7.3 ± 0.5	67.5 ± 1.5	99.5 ± 1.5
Lurcher male	5	72.4 ± 6.2[a]	15.6 ± 0.7[a]	42.8 ± 1.9[a]	11.0 ± 0.3[a]	5.0 ± 0.3[a]	60.4 ± 4.0	97.4 ± 3.0
Lurcher female	5	63.8 ± 4.7[a]	12.4 ± 1.1	36.2 ± 2.1[a]	9.6 ± 0.7[a]	4.8 ± 0.2[a]	55.8 ± 2.5	78.8 ± 3.0[a,b]

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

TABLE II. Body Weight and Muscle Weight in % of Body Weight in Normal and Lurcher Mice According to Sex

			Muscle weight (% of body weight)						
Group	n	Body weight (g)	Diaphragm	Anterior Temporalis	Masseter Superficialis	Anterior Digastric	Soleus	Tongue	Cardiac Ventricle
Normal male	5	30.4 ± 0.4	3.2 ± 0.3	0.8 ± 0.1	2.0 ± 0.2	0.5 ± 0.0	0.3 ± 0.0	2.1 ± 0.1	3.8 ± 0.3
Normal female	2	25.9 ± 1.1 [b]	3.7 ± 0.0	0.7 ± 0.1	2.1 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	2.6 ± 0.1[b]	3.8 ± 0.1
Lurcher male	5	24.3 ± 0.6 [a]	3.0 ± 0.3	0.6 ± 0.0 [a]	1.8 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	2.5 ± 0.2	4.0 ± 0.1
Lurcher female	5	20.3 ± 0.7 [a,b]	3.1 ± 0.2	0.6 ± 0.1	1.8 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	2.7 ± 0.1	3.9 ± 0.2

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

MHC 2A, 2X, and 2B and the slow type, MHC 1, except for S of normal females in which MHC 2B was not detected. 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 since 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). Whatever sex, the Dia of normal mice expressed predominantly MHC 2X. However, the female Dia presented more MHC 1 and 2A, and less MHC 2B than males. The male AT expressed predominantly MHC 2B (85%) whereas the female AT expressed mainly MHC 2X (48%) and 2B (45%). The male and female MS and AD expressed mainly MHC 2X (> 60%). Nevertheless, in these two muscles, the second major isoform was MHC 2B (> 26%) in male, and

TABLE III. Protein Concentration (μg per mg muscle) in Normal and Lurcher 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	56.8 ± 0.2	56.8 ± 0.2	50.3 ± 0.3	18.9 ± 0.0	51.3 ± 0.2	24.0 ± 0.1	35.4 ± 0.2
Normal female	44.9 ± 0.1[b]	40.0 ± 0.1[b]	42.1 ± 0.1[b]	36.1 ± 0.1[b]	50.9 ± 0.1[b]	34.4 ± 0.1[b]	26.3 ± 0.1[b]
Lurcher male	31.0 ± 0.0[a]	40.3 ± 0.1[a]	38.1 ± 0.2[a]	15.8 ± 0.0[a]	37.2 ± 0.1[a]	26.5 ± 0.0[a]	28.6 ± 0.1[a]
Lurcher female	58.6 ± 0.1[a,b]	29.9 ± 0.0[a,b]	12.2 ± 0.1[a,b]	14.9 ± 0.0[a,b]	50.1 ± 0.1[a,b]	27.8 ± 0.0[a,b]	22.1 ± 0.1[a,b]

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

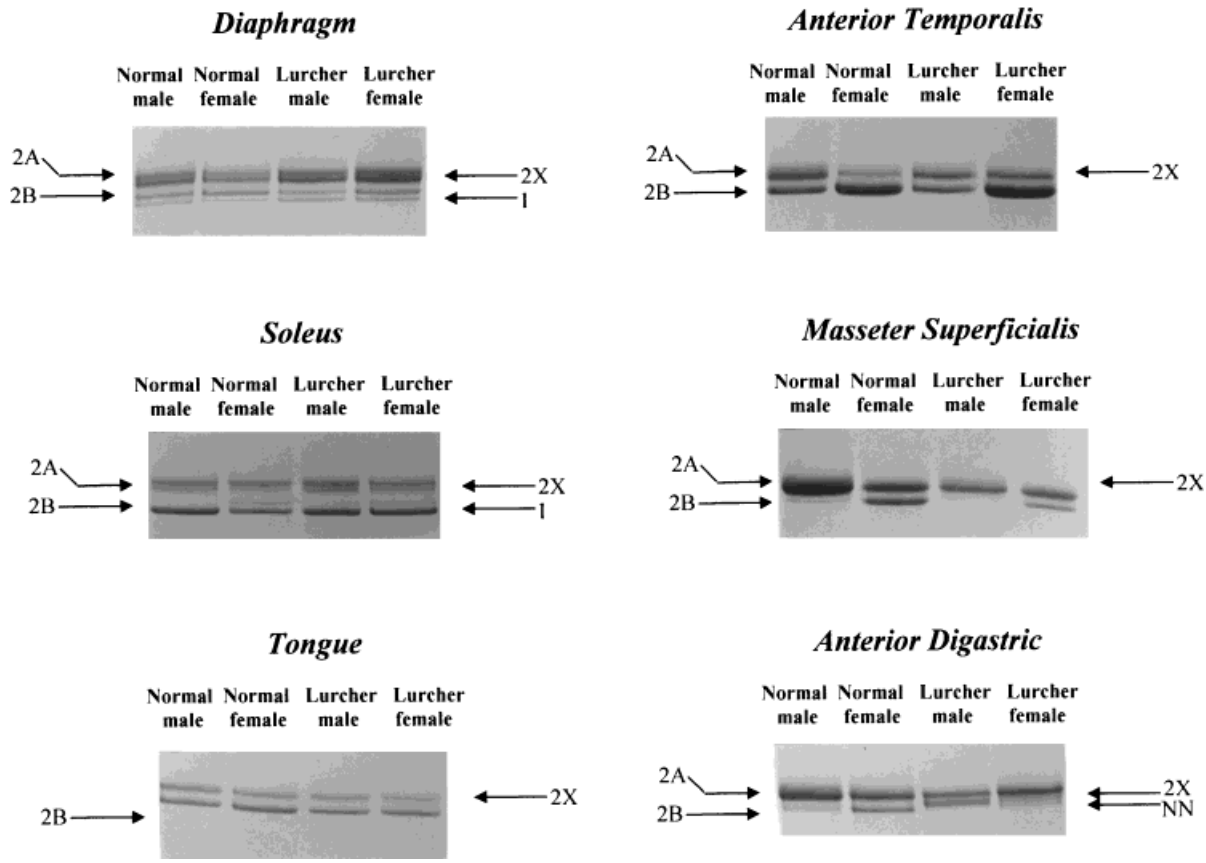

Fig. 1. MHC in six muscles of adult normal and Lurcher mice according to sex. Order of increasing electrophoretic mobility: adult fast 2A, adult fast 2X, NN, adult fast 2B and slow adult 1 types.

MHC 2A ($>14\%$) in female. The male MS presented even no MHC 2A at all. The female S profile was composed principally of MHC 1 (55%), while the male S expressed in equivalent proportions MHC 1 and 2A ($\sim 40\%$). In male T muscle, MHC 2B was the predominant isoform (64%), whereas in the female T, almost equal amounts of MHC 2X (45%) and 2B (55%) were present. In the second place, sex modified significantly the MHC relative expression in all the mutant muscles studied (Table IV). While the female and male mutant Dia expressed equal amounts of MHC 1 (5%) and 2X (47%), the male Dia expressed more MHC 2B and less MHC 2A than the female one. The male mutant AT expressed predominantly MHC 2B (79%), whereas the female one expressed equal amounts of MHC 2X and 2B (48%). In the MS muscle of both female and male Lc mice, MHC 2X was the predominant isoform (78–86%), whereas MHC 2A was only expressed in female and MHC 2B only in male. The male and female Lc AD both expressed principally MHC 2X ($>60\%$), the female muscle expressed nevertheless more MHC 2B and NN than the male. The major isoform in male and female Lc S was MHC 1 ($>48\%$), but the female S showed higher levels of MHC 2A and 2X and lower levels of MHC 2B and 1. In the mutant female and male T, the predominant isoform was MHC 2B, the female T expressed nevertheless more MHC 2X and less MHC 2B than the male.

Lc mutation affected the MHC relative expression both in male and female mice muscles. In Dia, the mutation led to a reduced expression of MHC 2B and an overexpression of MHC 1, 2A, and 2X, both in male and female. The Lc male AT muscle presented a lower expression of MHC 2B in aid of MHC 2A and 2X vs. normal muscle, whereas the Lc female AT muscle showed an increase of MHC 2B, a decrease of MHC 2A, and no change in MHC 2X levels. In MS, the mutation led to a disappearance of MHC 2B expression and no

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

	MHC, %				
Group	2A	2X	2B	1	NN
			Diaphragm		
Normal male	28.7 ± 0.2	45.1 ± 0.2	25.1 ± 0.2	1.2 ± 0.2	ND
Normal female	37.0 ± 0.4^{b}	45.0 ± 0.2	14.1 ± 0.2^{b}	4.0 ± 0.1^{b}	ND
Lurcher male	34.2 ± 0.4^{a}	47.2 ± 0.7^{a}	13.7 ± 0.2^{a}	4.9 ± 0.3^{a}	ND
Lurcher female	$38.7 \pm 0.5^{a,b}$	47.9 ± 0.3^{a}	$8.4 \pm 0.2^{a,b}$	5.0 ± 0.1^{a}	ND
			Anterior Temporalis		
Normal male	2.2 ± 0.1	13.3 ± 0.5	84.5 ± 0.5	ND	ND
Normal female	7.5 ± 0.5^{b}	47.5 ± 0.5^{b}	45.0 ± 0.4^{b}	ND	ND
Lurcher male	3.2 ± 0.2^{a}	18.0 ± 0.3^{a}	78.8 ± 0.3^{a}	ND	ND
Lurcher female	$4.6 \pm 0.4^{a,b}$	47.6 ± 0.3^{b}	$47.9 \pm 0.3^{a,b}$	ND	ND
			Masseter Superficialis		
Normal male	ND	61.9 ± 0.2	38.1 ± 0.2	ND	ND
Normal female	14.5 ± 1.0^{b}	83.9 ± 1.1^{b}	1.5 ± 0.1^{b}	ND	ND
Lurcher male	ND	77.8 ± 0.8^{a}	22.2 ± 0.8^{a}	ND	ND
Lurcher female	14.3 ± 1.2^{b}	85.7 ± 1.2^{b}	ND^{a,b}	ND	ND
			Anterior Digastric		
Normal male	6.3 ± 0.2	67.5 ± 0.3	26.2 ± 0.1	ND	ND
Normal female	17.1 ± 1.2^{b}	80.2 ± 1.2^{b}	2.7 ± 0.2^{b}	ND	ND
Lurcher male	ND^{a}	83.7 ± 0.5^{a}	4.1 ± 0.3^{a}	ND	12.2 ± 0.5^{a}
Lurcher female	ND^{a}	$9.6 \pm 0.9^{a,b}$	$12.5 \pm 0.6^{a,b}$	ND	$27.9 \pm 0.4^{a,b}$
			Soleus		
Normal male	42.9 ± 0.4	8.0 ± 0.3	8.1 ± 0.4	41.0 ± 0.2	ND
Normal female	34.1 ± 0.3^{b}	10.7 ± 0.4^{b}	ND^{b}	55.2 ± 0.3^{b}	ND
Lurcher male	34.2 ± 0.3^{a}	8.7 ± 0.5	4.5 ± 0.1^{a}	52.7 ± 0.5^{a}	ND
Lurcher female	$39.0 \pm 0.2^{a,b}$	$12.1 \pm 0.2^{a,b}$	$1.4 \pm 0.2^{a,b}$	$47.6 \pm 0.3^{a,b}$	ND
			Tongue		
Normal male	ND	36.1 ± 0.1	63.9 ± 0.1	ND	ND
Normal female	ND	45.0 ± 0.3^{b}	55.0 ± 0.3^{b}	ND	ND
Lurcher male	ND	29.9 ± 0.3^{a}	70.1 ± 0.3^{a}	ND	ND
Lurcher female	ND	$42.6 \pm 0.2^{a,b}$	$57.4 \pm 0.2^{a,b}$	ND	ND

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

change of MHC 2A and 2X in female, and an overexpression of MHC 2X with the detriment of MHC 2B in male. In male and female AD, the mutation led to the unexpression of MHC 2A and the expression of MHC NN. However, the male mutant AD presented a decrease in MHC 2B in aid of 2X, whereas it was the opposite in females. It should be noted that MHC NN was only detected in Lc female and male AD. The mutant male S muscle presented a lower level of MHC 2A and 2B and a higher level of MHC 1, whereas in female, MHC 2A and 2X were increased at the expense of MHC 1. Furthermore, MHC 2B which was undetected in normal female S, was expressed in mutant female S. The mutant T was affected in the same way in male and female, i.e. decrease in MHC 2X in aid of 2B.

DISCUSSION

In normal and mutant mice, the body weight was always greater in male than in female. Furthermore, the mutation was accompanied by a decrease in body weight. Muscle weight was related to sex in V and to phenotype in Dia, AT, MS, AD, S, and V. More precisely, in mutant mice, the male V muscles were heavier than female ones. The mutant muscles were lighter than normal ones, except in female AT, male and female T and male V, in which the same difference was observed but did not reach the significance level. Nevertheless, since a linear relation has been shown between skeletal muscle and body weight [Uchiyama et al., 1994], these variations can principally be attributed to body weight modifications. Indeed, in relative weight, these reductions were no more observable, except in mutant male AT. On the contrary, the relative weight of two muscles, T and V, tended to increase in mutant mice, and the normal female T was significantly greater than the male one. It appears therefore, that on one hand females are always globally smaller than males in both normal and Lc mice, and that on the other hand, the Lc mutation induces a

reduction in whole body growth but no specific muscle atrophy.

Protein concentration was considerably modified by sex and Lc mutation. The influence of sex on muscle protein content was different in normal and mutant mice. In normal mice, according to muscle, the protein concentration was lower in female in Dia, AT, MS, S, and V, and higher in AD and T. In mutant mice, the female showed a higher protein concentration in Dia, S, and T, whereas it was lower in the other muscles. Therefore, a general influence of sex on muscle protein concentration cannot be disclosed from these results. As regards Lc mutation, it induced a global reduction of muscle protein content, except for female Dia and for male T 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 authors evaluated, in rat skeletal muscles, the maximum velocity of shortening for the different fiber types, in muscle lengths per second: 0.639 ± 0.038 (mean $\pm$ 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 ones contained both MHC E, NN, and/or 2A, and finally, the fastest ones 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 V and female S, the muscles studied (Dia, AT, MS, AD, and T), which predominantly express the type 2 MHC isoforms, are fast-twitch muscles. The female S is a slow-twitch muscle, as it expresses nearly 55% of MHC 1. The V contains only MHC α, which is a fast MHC too [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 authors showed that mouse new-born heart contained MHC α and β but that MHC β was only present during development and disappeared soon after birth.

While in hybrid fibers, shortening velocity is non-linearly related to MHC isoform composition, among these muscles, 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 muscle, expressing predominantly 2X (45%) but also approximately 25% of 2A and 2B, which in turn is faster than the S muscle, in which MHC 2A and 1 each one represent 40%. On the other hand, in female normal mice, T is the fastest muscle, with 55% of MHC 2B, followed by AT (MHC 2B = 45%), which is faster than MS, AD (expressing principally MHC 2X) and Dia (MHC 2A = 37% and 2X = 45%) and finally S, the slowest one in which MHC 1 represents 55%. The classification of muscles in terms of shortening velocity is, then, almost similar in normal male and female. All the studied normal female muscles are slower than male ones. It seems therefore that sexual dimorphism is muscle-type unspecific, contrary to the observations of Hartmann et al. [1999] in another mouse strain (B6C3-a/a) in which the sexual dimorphism was muscle-type specific. This muscle sexual dimorphism appears then strain-specific. However, in the present study, some normal male and female muscles are very different as regards MHC isoform expression. In male, the AT muscle expresses predominantly MHC 2B, whereas in female, it expresses as much MHC 2X as 2B. MHC 2A is only expressed in female MS, and MHC 2B is only expressed in male S. Eason et al. [2000] reported a similar sexually dimorphic expression of MHC in the adult mouse MS.

On the other hand, in mutant male mice, the AT and T muscles, containing mainly type 2B (> 70%), are faster than the AD and MS muscles, containing mainly 2X (> 77%), which in turn are faster than the Dia muscle, in which MHC 2A and 2X represent 81%, the slower muscle being S which contains 52% of MHC 1. On the contrary, in female mutant mice, T is the fastest muscle, with 57% of MHC 2B, followed by AT (MHC 2B = 48% and 2X = 48%), which is faster than MS and AD (expressing mainly MHC 2X), followed by Dia (expressing principally MHC 2X and 2A) and finally S, the slowest one in which MHC 2A and 1 represent 39% and 48% respectively. As in normal mice, the classification of muscles in terms of shortening velocity is not very different in mutant male and female. Globally, once again as in normal mice, Lc female muscles are slower than male ones, except for the AD muscle which is approxi-

mately as rapid as the male one and the S muscle which is faster in female.

Apart from V, Lc mutation affected significantly MHC relative expression in the six muscles studied, in male and female mice. First, in male muscles, except for T, mutation led to a significant decrease in fast MHC 2B in aid of slower MHCs: MHC 2A and 2X in AT, MHC 2X in MS, MHC 2X and NN in AD, MHC 2A, 2X and 1 in Dia and MHC 1 in S (accompanied by a decrease in MHC 2A). These five muscles were then slower in mutant males than in normal ones. On the contrary, the T muscle of mutant males, which showed higher levels of MHC 2B and lower levels of MHC 2X, became faster than that of normal ones. Secondly, in female muscles, MHC 2B also decreased in Dia and MS, but it was accompanied by an increase in MHC 2A, 2X, and 1 in Dia. MHC 2B increased in four muscles, to the detriment of MHC 2A in AT, MHC 2X in AD and T, MHC 1 in S. Contrary to male muscles, the only female mutant muscles which became slower than normal ones were the Dia. In spite of significant differences in MHC isoforms relative expression, the female mutant MS and AD muscles were comparable to normal ones in terms of shortening velocity. Finally, the female mutant AT, S, and T muscles were faster than female normal ones. The muscular changes accompanying mutation were then very different in males and females. Consequently, the modifications induced by Lc mutation on muscle structure are sex-dependent as well as muscle type-specific.

Contrary to male, the female mutant S showed more MHC 2A, 2X, and 2B and less MHC 1 than the female normal one and became faster than normal female S. With the opposite of the other muscles studied, the female mutant S was faster than the male one. The S muscle seems, therefore, to be affected in peculiar manner by sex and phenotype. It altered in the opposite direction with regard to the other muscles: S was slower in normal female than in male, and faster in mutant female than in male, whereas Dia, AT, MS, and T were slower in these two cases, and AD was slower in the first case and approximately equally rapid in the second one.

Now, 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 fatigable (MHC 2X and 2B or 2B alone). Furthermore, Zhan et al. [1998] observed a slower cross-bridge cycling rate and lower ATP consumption of fibers expressing the MHC NN isoform, together with a higher oxidative capacity, which may lead to a better balance between energy supply and utilization and thus a lower susceptibility to fatigue than fibers expressing MHC 2. As the Lc mutation decreased the relative proportion of the fastest MHCs to the aid of the slowest MHCs in all male muscles except T and V (no difference), this transition was accompanied by increase in male muscle fatigue resistance. The mutant male T was less fatigue resistant than the normal one, since MHC 2B increased at the expense of MHC 2X. On the contrary, in females, the mutant Dia, MS, and AD became more fatigue resistant than normal ones, whereas AT, S, and T were more fatigable. In AT and S, the Lc mutation effect is then sex-dependent.

In summary, the Lc mutant mice showed a reduced whole body growth but not exclusively muscle atrophy. Furthermore, except for female Dia and male T, the Lc mutation was accompanied by a global decrease in muscle protein content which makes muscles more fragile. The remaining muscle fibers have then to work harder. The male increased fatigue resistance could therefore constitute an adaptative 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 favoring predominant expression of slower MHC isoforms. However, this hypothesis cannot explain entirely the fatigability changes in female mutant muscles, since AT, S, and T were more fatigable than normal ones. To our knowledge, it is the first time that a mutation is accompanied by a sex-dependent muscle fatigue resistance modification. Actually, in dystonic mutant mice, Hartmann et al. [1999] observed an increased fatigue resistance in male and female, except for the S which was more fatigable. S, a continually active postural muscle, is faster and consequently less fatigue resistant in Lc female mice. This female lower fatigability resistance could be in part linked to the Lc mutant hypoactivity described by Fortier et al. [1987].

OPCA patients experience ataxia, dysarthria, muscle spasms or weakness, and reduction or

slowness of movements [Gilman and Quinn, 1996; Wenning et al., 1996]. Our results suggest that these symptoms could be attributable, in part, to the reduced fatigue resistance of lower limb postural muscles, observed in female Lc. 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]. Now Lc mutant mice exhibit motor control deficits [Martin and Caddy, 1977], then the slower MHC profiles observed in some Lc mice muscles could be explained by these deficits.

On the other hand, the modifications observed in masticatory and respiratory muscles of Lc mutant mice could contribute to identify the origin of OPCA dysarthria. Respiratory failure is the most common cause of death in OPCA patients [Kurisaki, 1999]. Some patients deaths seem related to some problems of respiratory center such as central chronic respiratory failure. The Dia of Lc mutant mice is slower than normal one, this slow-twitch modification resembles denervation effect [d'Albis et al., 1995]. Our results could then corroborate a respiratory center dysfunction. In Lc mutant mice, the muscle contractile properties are modified according to muscle type and sex. To our knowledge, there is no symptomatic study which among OPCA patients distinguishes the men from the women, it is the same in Lc mice studies. The differences observed between male and female Lc suggest that it would be interesting to take into account the sex factor in further studies devoted to OPCA disease or Lc mutation. Further experiments, especially ontogenic studies, are now required to precise the respective roles of sensory and motor denervation and altered loading in development and structure of Lc mutant mice muscles and to contribute to a better understanding of OPCA.

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