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Elemental analysis of biological tissues of Dmd^{mdx}/J and C57BL/6J mice strains investigated by neutron activation analysis

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Abstract In order to understand in more details the alterations that Duchenne muscular dystrophy disease may cause in biological tissues (blood, tibia, quadriceps and heart), correlations matrixes of the Dmd^{mdx}/J dystrophic mice as well as C57BL/6J (control group) were generated. These mice were obtained from Jackson Laboratory (Maine, USA) and bred at IPEN (Dmd^{mdx}/J), and at Centro de Estudos do Genoma Humano (C57BL/6J), both research centers at São Paulo city. Elements of clinical and nutritional relevance (Br, Ca, Cl, K, Mg, Na and S) were investigated by neutron activation analysis. These measurements were performed using the nuclear reactor IEA-R1 (3.5–4.5 MW, pool type) at IPEN. Comparisons between concentrations and correlations in these biological tissues, of these strains, showed that a Ca and Mg in blood are altered for the dystrophic mice. A significant change in the heart of dystrophic mice was also observed suggesting that a constant monitoring is required. Moreover, these results may help the researchers to evaluate the efficiency of new treatments and to compare the advantages of different treatment approaches before performing tests in patients with muscular dystrophy.

Keywords Neutron activation analysis · Duchenne muscular dystrophy · Biological tissues · Clinical analysis · Correlation matrixes

Introduction

Duchenne muscular dystrophy (DMD) [1–3] is the most severe and prevalent type of muscular dystrophy. It is caused by a mutation of the dystrophin gene due to the absence of the protein dystrophin in muscles [4, 5]. In individuals with DMD this protein is embedded in the DMD gene. This gene has 79 exons (the largest of our body) fitted together like pieces of a jigsaw puzzle and form the genetic code for the dystrophin protein [6]. Duchenne patients have mutations in their DMD gene. The most common mutation is when one exon or more are missing from the gene (as called deletion) [1]. The disorder is caused by a mutation in the dystrophin gene. Unlike most genes, which come in pairs in both sexes and stay active throughout life, the dystrophin gene is located in humans on the X chromosome. Since males have only one X chromosome this disorder usually affects boys much more than girls [1–3].

Considering that the blood is responsible for transportation, regulation and protection of the body, the functions performed by its circulation in biological tissues (both organs and bones) are essential to the proper functioning of the body and to act as an indicator in case of malfunctions [7]. Based on this, in the previous studies, we have investigated blood of dystrophic mice (genetically modified) such as: A/J, Dmd^{mdx}/J and SJL/J [8–12], using

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Table 1 Concentrations of Br, Ca, Cl, K, Mg, Na and S elements in biological tissues of Dmd^{mdx}/J and C57BL/6J (CG) mice strains

Elements	Tibia (g kg^{-1})	Blood (g L^{-1})	Quadriceps (g kg^{-1})	Heart (g kg^{-1})
Br	nd	5.6 ± 0.6^{CG}	0.32 ± 0.09^{CG}	1.31 ± 0.19^{CG}
		3.7 ± 0.2^{m}	0.49 ± 0.08^{m}	1.33 ± 0.27^{m}
		3.8 ± 0.3^{f}	0.46 ± 0.11^{f}	1.04 ± 0.25^{f}
Ca	129.72 ± 6.28^{CG}	0.289 ± 0.066^{CG}	0.061 ± 0.016^{CG}	0.035 ± 0.015^{CG}
	126.41 ± 9.58^{m}	0.244 ± 0.085^{m}	0.070 ± 0.026^{m}	0.028 ± 0.011^{m}
	121.80 ± 15.01^{f}	0.156 ± 0.034^{f}	0.060 ± 0.022^{f}	0.019 ± 0.009^{f}
Cl	1.20 ± 0.13^{CG}	3.13 ± 0.26^{CG}	0.54 ± 0.05^{CG}	1.33 ± 0.38^{CG}
	1.11 ± 0.10^{m}	2.71 ± 0.18^{m}	0.59 ± 0.09^{m}	1.62 ± 0.23^{m}
	1.43 ± 0.16^{f}	2.99 ± 0.28^{f}	0.67 ± 0.07^{f}	1.24 ± 0.20^{f}
K	nd	2.45 ± 0.48^{CG}	5.13 ± 0.48^{CG}	2.82 ± 0.54^{CG}
		2.39 ± 0.32^{m}	4.90 ± 0.51^{m}	2.54 ± 0.38^{m}
		2.14 ± 0.43^{f}	4.82 ± 0.51^{f}	2.94 ± 0.31^{f}
Mg	2.11 ± 0.28^{CG}	0.027 ± 0.009^{CG}	0.274 ± 0.022^{CG}	0.163 ± 0.025^{CG}
	2.46 ± 0.37^{m}	0.017 ± 0.002^{m}	0.222 ± 0.019^{m}	0.156 ± 0.022^{m}
	2.12 ± 0.38^{f}	0.010 ± 0.003^{f}	0.226 ± 0.019^{f}	0.152 ± 0.028^{f}
Na	3.50 ± 0.47^{CG}	2.37 ± 0.25^{CG}	0.59 ± 0.06^{CG}	1.06 ± 0.28^{CG}
	3.78 ± 0.33^{m}	2.38 ± 0.20^{m}	0.60 ± 0.08^{m}	1.11 ± 0.14^{m}
	3.73 ± 0.55^{f}	2.46 ± 0.32^{f}	0.66 ± 0.08^{f}	1.06 ± 0.16^{f}
S	nd	1.17 ± 0.22^{CG}	2.44 ± 0.46^{CG}	3.02 ± 0.42^{CG}
		1.02 ± 0.17^{m}	2.17 ± 0.54^{m}	2.25 ± 0.47^{m}
		0.92 ± 0.16^{f}	2.06 ± 0.44^{f}	2.27 ± 0.67^{f}

CG control group, *m* male, *f* female, *nd* not determined

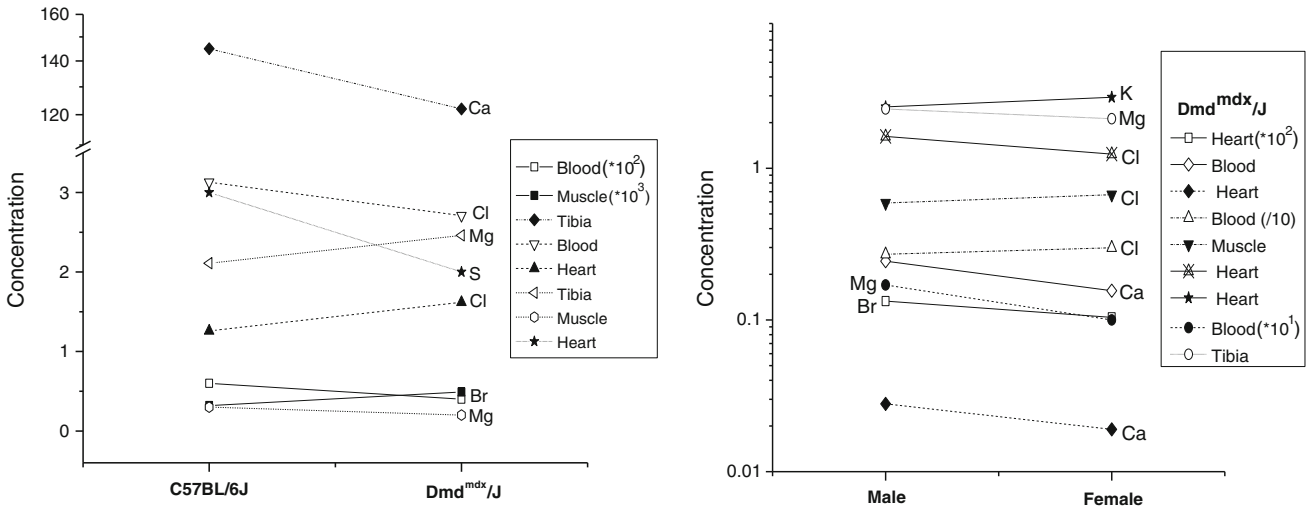

Fig. 1 Student *t* distribution between control and dystrophic male groups, and between male and female dystrophic groups

neutron activation analysis technique. In this work, we show the correlation matrixes in biological tissues (whole blood, bones and organs) of Dmd^{mdx}/J (dystrophic male and female group) and C57BL/6J (control male group) mice in attempt to identify the physiological differences through a comparison among these groups and to provide more information in the understanding of the DMD.

Experimental

Blood and organs of Dmd^{mdx}/J male ($n = 10$) and female ($n = 10$) and C57BL/6J (male = 16) were collected according to the rules approved by the IPEN Ethical Committee in Animal Experimentation (087/99). The measurements of the NAA were performed

Table 2 Correlation coefficients between Ca, Cl, Mg and Na concentrations in tibia of C57BL/6J and Dmd^{mdx}/J (male and female) mice strains

Tibia	Ca	Cl	Mg	Na
C57BL/6J				
Ca	**1**	0.32	−0.13	**0.57**
Cl		**1**	0.15	0.26
Mg			**1**	0.31
Na				**1**
Dmd^{mdx}/J (male)				
Ca	**1**	0.06	−0.03	**0.64**
Cl		**1**	−0.11	0.14
Mg			**1**	0.16
Na				**1**
Dmd^{mdx}/J (female)				
Ca	**1**	0.00	0.66	**0.84**
Cl		**1**	0.11	0.32
Mg			**1**	0.41
Na				**1**

Bold values represent main correlations

using the nuclear reactor IEA-R1 (3.5–4.5 MW, pool type) at IPEN. More details about samples preparation, nuclear instrumentation, methods of analysis and the results of quality control are presented in a previous study [8].

The Ca, Cl, K, Mg and Na elements were investigated due to the relevance for evaluation of electrolytic and nutritional disorders which has high prevalence in patients with DMD [13]. Br and S were also investigated: Br, since the bromides are usually present in Brazilian diet (mainly sea food) as well in medications prescriptions and drugs, highly consumed by Brazilian population (according to the last census conducted by ANVISA [14] and CONAD [15, 16]) and S, due to its use as fertilizer (elemental-S) in tropical soil (case of Brasil) [17] that can increase the levels of sulfur in the body of these patients by a diet rich in vegetables and fruits [18, 19]. Moreover, according to the study by Santos [20], the nutritional management of patients with DMD has been emphasized. In milder cases, a diet rich in fruits and vegetables is essential for controlling the levels of electrolytes (especially Br, Ca, Na and S), contributing to health life quality of these patients.

Results and discussion

The concentrations of the elements investigated in blood, bone and organs are summarized in Table 1 and they are in

Table 3 Correlation coefficients between Br, Ca, Cl, K, Mg, Na and S concentrations in whole blood of C57BL/6J and Dmd^{mdx}/J (male and female) mice strains

Whole Blood	Br	Ca	Cl	K	Mg	Na	S
C57BL/6J							
Br	**1**	−0.32	0.45	−0.09	0.16	**0.53**	0.34
Ca		**1**	−0.18	0.17	0.20	−0.30	−0.04
Cl			**1**	−0.01	0.03	0.35	−0.18
K				**1**	0.20	−0.21	**0.55**
Mg					**1**	−0.01	0.05
Na						**1**	0.01
S							**1**
Dmd^{mdx}/J (male)							
Br	**1**	0.05	0.44	−0.05	−0.42	0.31	0.03
Ca		**1**	0.53	0.65	0.26	0.16	0.14
Cl			**1**	**0.78**	−0.02	**0.77**	0.39
K				**1**	0.19	0.36	0.25
Mg					**1**	−0.19	0.16
Na						**1**	0.53
S							**1**
Dmd^{mdx}/J (female)							
Br	**1**	0.23	0.57	0.09	0.42	0.50	−0.21
Ca		**1**	0.22	0.01	0.02	0.43	−0.43
Cl			**1**	−0.30	**0.71**	**0.96**	−0.05
K				**1**	−0.17	−0.38	−0.40
Mg					**1**	0.60	−0.14
Na						**1**	−0.14
S							**1**

Bold values represent main correlations

Table 4 Correlation coefficients between Br, Ca, Cl, K, Mg, Na and S concentrations in quadriceps of C57BL/6J and Dmd^{mdx}/J (male and female) mice strains

Muscle	Br	Ca	Cl	K	Mg	Na	S
C57BL/6J							
Br	**1**	0.08	**0.86**	0.56	−0.09	**0.90**	−0.13
Ca		**1**	0.10	0.28	−0.33	0.15	0.51
Cl			**1**	0.59	−0.12	**0.88**	−0.07
K				**1**	−0.46	0.57	0.65
Mg					**1**	−0.05	−0.46
Na						**1**	−0.14
S							**1**
Dmd^{mdx}/J (male)							
Br	**1**	−0.48	−0.46	−0.05	**0.80**	−0.39	0.57
Ca		**1**	0.42	0.32	−0.13	0.50	−0.09
Cl			**1**	0.21	−0.48	0.40	−0.07
K				**1**	−0.17	0.34	0.54
Mg					**1**	−0.03	0.20
Na						**1**	−0.23
S							**1**
Dmd^{mdx}/J (female)							
Br	**1**	−0.01	0.07	0.08	0.42	0.06	−0.40
Ca		**1**	−0.03	0.03	−0.18	−0.12	−0.15
Cl			**1**	0.57	−0.11	**0.95**	0.54
K				**1**	0.00	0.63	0.59
Mg					**1**	−0.08	−0.55
Na						**1**	0.58
S							**1**

Bold values represent main correlations

agreement with the data obtained in previous studies [8, 9]. The significance of differences between control and dystrophic male groups as well as between gender (male and female dystrophic mice), assessed by Student's t test ($p < 0.05$), are presented in Fig. 1. The correlation matrixes for C57BL/6J (control group) and Dmd^{mdx}/J (dystrophic male and female groups), for tibia, blood, quadriceps and heart, are shown in Tables 2, 3, 4, and 5, respectively. The main correlation coefficients are highlighted.

The concentration results for Br in blood for Dmd^{mdx}/J males (Table 1), is below the lower limit when compared to control group (4.4 mg L^{-1}), considering a confidence interval of 95 % usually adopted in clinical practice. Moreover, the changes in blood levels of Br ($p < 0.05$, Fig. 1) suggest a dependence that can be directly related to their diet once the strains (C57BL/6J and Dmd^{mdx}/J) were generated in different places (Human Genome Research Center and IPEN) and subjected to different feeds. With regard to the gender for Ca ($p < 0.05$), Cl ($p < 0.05$) and Mg ($p < 0.05$), decreased concentrations in females was observed. Although it is not possible to compare dystrophic female mice with the control group (composed only by males), these variations in the element concentrations suggest that blood can be a good indicator for monitoring and evaluation of the DMD progression.

In Fig. 2, the ratios between mean value of Dmd^{mdx}/J and C57BL/6J ($C_{DMD}/C_{control}$) and Dmd^{mdx}/J Gender (C_{Female}/C_{Male}) in whole blood are presented. The behavior of these ratios showed that the blood levels of dystrophic mice were lower, emphasizing the relevance of blood analyses; exceptions were Na ($C_{DMD}/C_{control}$), K and S (C_{Female}/C_{Male}).

Correlations between genders were also different. A strong correlation of Cl with Mg in female mice was observed, but for male (dystrophic and control) this correlation did not exist. Similarly, while Cl and K are strongly correlated in blood for dystrophic male mice, this correlation did not exist in blood for control mice and in dystrophic females the results showed an inverted behavior (−0.30).

Related to the concentrations in other organs and bone, the concentration and correlation data, mainly in heart (Tables 1 and 5, respectively), were substantially altered between control and dystrophic male groups as well as between gender (male and female dystrophic mice)

Table 5 Correlation coefficients between Br, Ca, Cl, K, Mg, Na and S concentrations in heart of C57BL/6J and Dmd^{mdx}/J (male and female) mice strains

Heart	Br	Ca	Cl	K	Mg	Na	S
C57BL/6J							
Br	**1**	0.38	−0.18	0.03	−0.41	−0.01	0.09
Ca		**1**	0.18	0.00	−0.48	0.16	0.56
Cl			**1**	0.28	−0.25	**0.92**	0.44
K				**1**	0.13	0.56	−0.15
Mg					**1**	−0.31	−0.52
Na						**1**	0.34
S							**1**
Dmd^{mdx}/J (male)							
Br	**1**	0.69	0.41	0.36	−0.23	0.23	−0.25
Ca		**1**	−0.16	0.19	−0.42	0.18	−0.17
Cl			**1**	−0.17	0.01	−0.01	0.06
K				**1**	0.03	0.34	0.24
Mg					**1**	−0.34	0.41
Na						**1**	0.17
S							**1**
Dmd^{mdx}/J (female)							
Br	**1**	0.54	0.49	−0.26	−0.31	0.55	0.07
Ca		**1**	−0.21	−0.35	−0.26	−0.27	−0.45
Cl			**1**	−0.12	−0.12	**0.94**	0.61
K				**1**	−0.05	−0.03	0.16
Mg					**1**	−0.27	−0.23
Na						**1**	0.59
S							**1**

Bold values represent main correlations

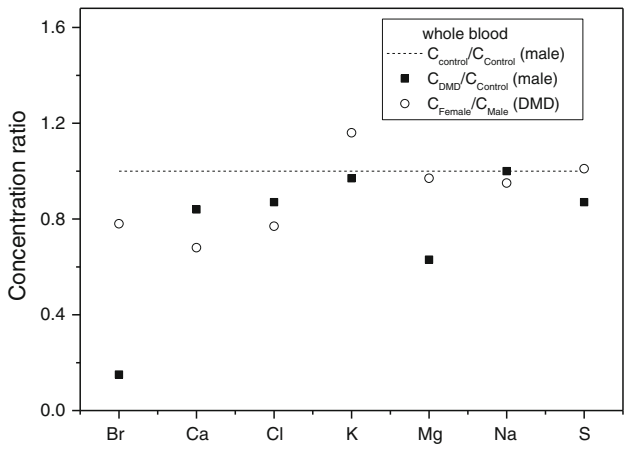

Fig. 2 Mean ratios between mean value of Dmd^{mdx}/J and C57BL/6J ($C_{DMD}/C_{control}$) and Dmd^{mdx}/J gender (C_{Female}/C_{Male}) in whole blood

suggesting that the heart status must be constant monitoring in DMD subjects.

Conclusions

The alteration in some correlation coefficient data among the elements in control and disease status indicates a connection between these elements in whole blood, tibia, quadriceps and heart. The comparison between concentration and correlation data in blood emphases the need of periodic clinical analysis of Ca and Mg as well as the heart status constant monitoring.

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