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Ions concentration in blood samples of SJL/J dystrophic mice strains using X-ray fluorescence spectrometry

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Abstract. Star This study proposes an investigation of ions in whole blood of the dystrophic animal model SJL/J (mice strain with dysferlin protein deficiency) and in the control group (C57BL/6J) using the Energy Dispersive X-ray Fluorescence (EDXRF) spectrometry technique. The comparison between control and dystrophic mice results shown an increase in blood for P, S, K and Fe ($p < 0.05$) while a decrease in Ca ($p < 0.05$). This elemental analysis will contribute to evaluate the best diagnostic, care and treatment procedures, for the Progressive Muscular Dystrophy.

1. Introduction

The Progressive Muscular Dystrophies (PMDs) are genetic diseases that affect muscular groups. More than thirty different forms of muscular dystrophies have been identified and, all of them have a hereditary character and has no cure [1-3]. This study proposes an investigation of ions of clinical relevance (such as, Ca, Cl, Cu, K, Fe, P and S) in whole blood of the dystrophic animal model (SJL/J mice strain - dysferlin protein deficiency) [4,5], using the Energy Dispersive X-ray Fluorescence (EDXRF) technique. The advantages of using this alternative procedure are the facilities that can be introduced, that is, the measurements can be carried out in a few minutes using small amounts of blood (few microliters), which is an important abridgment when the biological material is scarce. The conventional procedure performed in serum (using at least a 1.0 mL)[6], which requires the euthanize of this small animal model (the total mice body blood ~ 1.2 mL). Blood analyses was also performed in a healthy mouse strain (C57BL/6J), adopted as control group, with the intent to check differences among these groups.

2. Experimental Procedure

The mice strains came from Jackson Laboratory (Maine, USA) [5] and were later inbred at IPEN (São Paulo, Brasil). Whole blood samples from SJL/J (n=6) and C57BL/6J (n = 12) male mice were collected. The blood collection was performed according to the rules approved by the Animal Research Ethics Committee (087/99). For sample preparation aliquots of 50 μL whole blood (duplicate), from each animal model collected by the retro-orbital venous plexus, were transferred to filter paper (Whatman, nº 41) using a calibrated micropipette. The EDXRF analyses were performed using a portable XRF Spectrometer (PXRFS), composed by an Amptek detector X-123 SDD and an X-ray tube with Ag target.

The characteristic of the fluorescence intensity of K_α line was measured with a Si Drift detector (25 mm^2 x 500 μm) with Be window (12.5 μm). Each biological sample was irradiated for 300 to 900 s using 5μA and 30kV. The analyses were performed using WinQxas software program (IAEA, version 1.3).

3. Results and discussion

For the PXRFS calibration, standard solutions for each reference control sample, containing varying concentrations were prepared. For example, the calibration curve for Fe Kα line is presented in Figure 1.

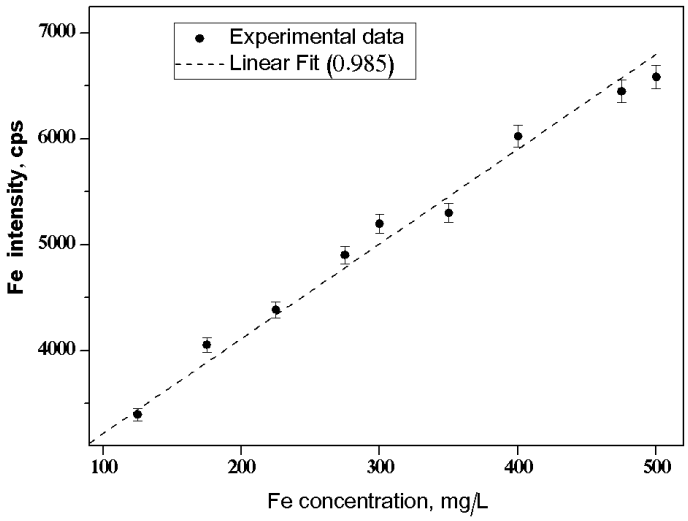

Figure 1. Calibration curve for Fe Kα line

The results for elements concentrations in whole blood mice strains samples using EDXRF technique are shown in Table 1. The results were expressed by mean value (MV), standard deviation (±1SD) and by the range for a confidence interval of 95% usually adopted in clinical evaluation. To visualize, in Figure 2 are shown the results (duplicate) of SJL/J species whole blood by using EDXRF analysis. A comparison between control and dystrophic animal models results are presented in Figure 3

Table 1. Elements concentrations in whole blood by EDXRF analysis

	SJL/J n=6	C57BL/6J n=12
Elements	MV ± 1SD [range]	
P, mg/L	1192 ± 243 [706 – 1678]	781 ± 185 [411 – 1051]
S, mg/L	1519 ± 312 [895 – 1863]	1229 ± 200 [829– 1629]
Cl, mg/L	2933 ± 380 [2173 – 3693]	2954± 442 [2070 – 3838]
K, mg/L	2488 ± 283 [1922- 3054]	2001± 513 [975 – 3027]
Ca, mg/L	142 ± 61 [20 – 134]	186±20 [146 – 226]
Mn, μg/L	0.68 ± 0.10 [0.48 – 0.88]	nd

Fe, mg/L	324 ± 29	187± 13
	[266 – 382]	[161 – 213]
Cu, µg/L	3.90± 0.45	3.44 ± 0.33
	[3.00 - 4.80]	[2.78 – 4.10]

n: number of samples
nd: not determined

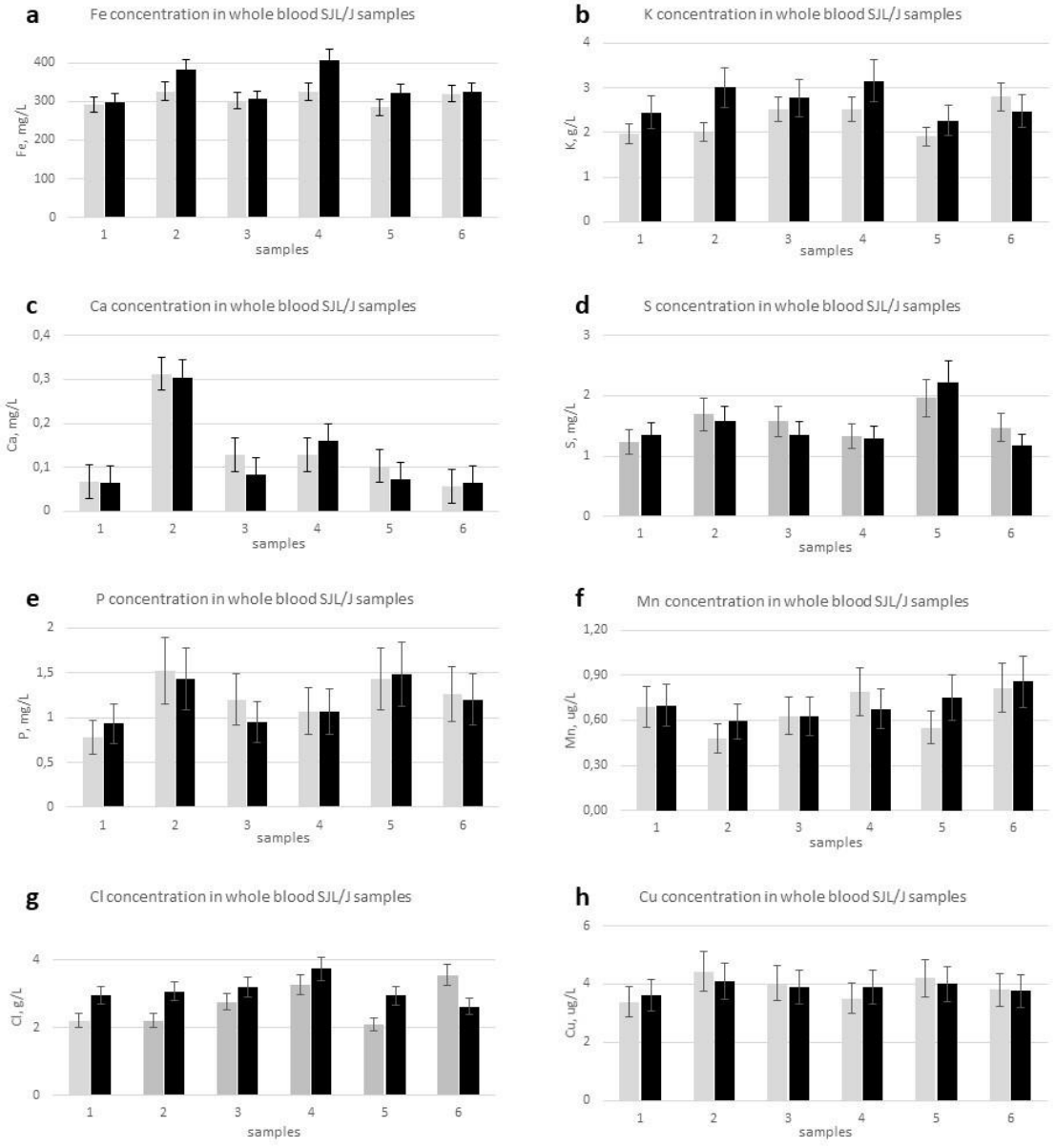

Figure 2. Elements Fe (a), K(b), Ca(c), S(d), P(e), Mn(f), Cl(g) and Cu(h) concentration for SJL/J whole blood mice samples analyzed in duplicate

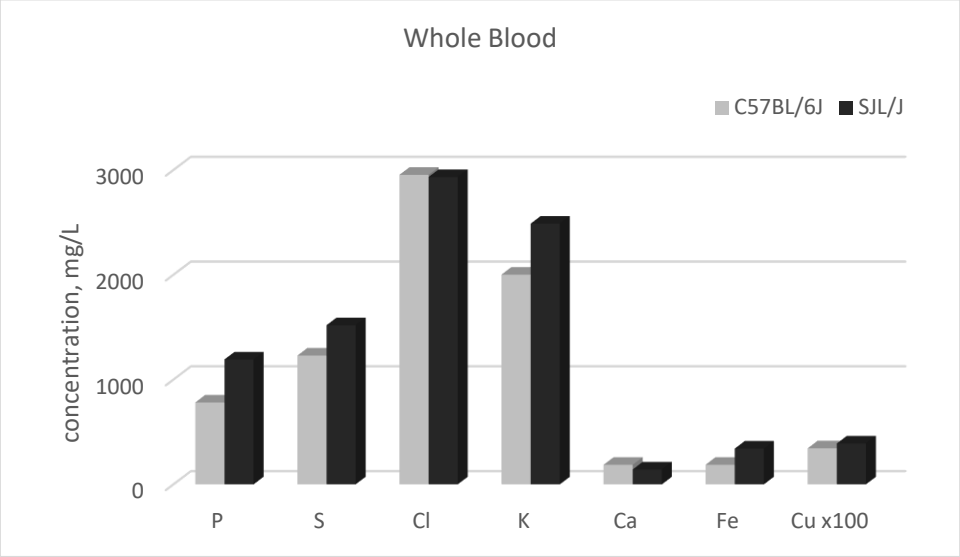

Figure 3. Whole blood concentrations results for SJL/J & C57BL/6J

By establishing an indicative interval for ions in whole blood of SJL/J dystrophic mice strain enable to verify differences among them (dystrophic and control), which is an important condition to the animal model selection in the investigation of muscular dystrophy. According to t-test, there is agreement between the species only for Cl. The comparison between control and dystrophic mice emphasizes an increase in blood for P, S, K and Fe ($p < 0.05$) while a decrease in Ca is more accentuated ($p < 0.05$) in the absence of dysferlin protein.

4. Conclusion

The elements concentration in whole blood for the SJL/J dystrophic animal model emphasize some significant differences for several ions when compared with the control group (C57BL/6J). Besides, the study of ions behavior in in whole blood this dystrophic specie contribute for a more accurate evaluation on the diagnoses.

5. Acknowledgments

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References

[1] Emery A E H 2002 *The Lancet* **359** 687. doi:10.1016/S0140-6736(02)07815-7.
[2] Zatz M 2005 *Science* **308** 55.
[3] Chelly J and Desguerre I 2013 *Handb Clin Neurol* **113** 1343. doi:10.1016/B978-0-444-59565-2.00006-X.
[4] Blake D J,Weir A,Newey S E and Davies K E 2002 *Physiol Rev* **82** 291. doi:10.1152/physrev.00028.2001.
[5] SJL/J dystrophic mice strain - Jackson Laboratory, https://www.jax.org/strain/000686.
[6] Zamboni C B,Azevedo M R and Metairon S 2018 *In* Raio-X para dosagem de ferro no sangue: Prática Clínica *Novas Edições Acadêmicas* Mauritius p.^pp. 19