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PAPER

Natural variations of lithium isotopes in a mammalian model

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Despite lithium's extensive clinical applications, the cellular and molecular basis for the therapeutic effects remains to be elucidated. The large difference in mass between the two lithium isotopes (^{6}Li and ^{7}Li) has prompted biochemists to explore the metabolism of Li by using pure ^{6}Li and ^{7}Li labeled drugs. However, experiments were carried out at very high Li concentrations, which did not reflect natural conditions. In the present study, we consider, for the first time, the natural variations of the ^{7}Li/^{6}Li ratio in the organs and body fluids of an animal model, sheep. Each organ seems to be characterized by a specific Li isotope composition. So far, the range of the ^{7}Li/^{6}Li ratio in the sheep body, expressed as δ permil variations relative to the L-SVEC standard (δ^7Li), is about 40‰, between muscles (~40‰) and kidney (~0‰). Relative to a dietary δ^7Li value of ~+17‰, serum, red blood cells, muscle, liver, brain and kidney have a ^{7}Li enrichment of −12‰, −14‰, +22‰, +5‰, −3‰ and −15‰, respectively. The Li isotope composition is likely to be fractionated during intestinal absorption, with a greater absorption of ^{6}Li relative to ^{7}Li. According to previous conclusions obtained with ^{6}Li and ^{7}Li labeled chemicals, ^{6}Li appears to diffuse into erythrocytes faster than does ^{7}Li. However, this does not hold for myocytes and hepatocytes, because these two tissues have a higher δ^7Li level than serum. Purely diffusive isotopic fractionation would leave all organs ^{7}Li-depleted relative to the serum, which is not the case, suggesting that active, molecule-specific, isotopic fractionation occurs in the body. Our preliminary results suggest that natural Li isotope variations can shed light on its regulation in the body, being active or passive.

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Introduction

In 1949, John Cade introduced lithium (Li) in psychiatric medicine due to its ability to counter bipolar disorders.[1] Less than a quarter of a century later, one estimation claims that the use of Li has saved $145 billion in hospitalization costs related to manic illnesses in USA.[2] More recently, Li has been found to delay the evolution of neurodegenerative diseases,[3] such as Alzheimer's,[4] Parkinson's,[5] Huntington's,[6] and amyotrophic lateral sclerosis.[7] Modern hypotheses for the therapeutic effect of Li postulate that Li competes with magnesium (Mg), due to similar ionic radii,[8] in inhibiting several Mg-dependent enzymes involved in specific neuro-transmission pathways including cAMP,[9] IMPase[10] and GSK-3β.[11] Earlier studies instead focused on the relative effects of pure ^{6}Li and ^{7}Li and on natural ^{7}Li/^{6}Li to highlight the therapeutic mechanism of Li.[12–15] *In vitro* experiments of incubation of blood with pure ^{6}Li or ^{7}Li revealed a higher rate of transport of ^{6}Li into erythrocytes than for ^{7}Li.[12] In mice, toxic doses of Li were proved to be more lethal when administered as pure ^{6}Li compared to equivalent doses of natural ^{7}Li/^{6}Li and pure ^{7}Li.[13] After subcutaneous administration of equimolar ^{7}Li/^{6}Li, rat brains exhibit a ^{7}Li/^{6}Li ratio of 0.66.[14] In cats fed orally with pure ^{6}Li, cerebrospinal fluid and plasma have higher Li concentrations than when they are given the same dose of pure ^{7}Li.[15] All these results clearly demonstrate that ^{6}Li is much more biologically reactive than ^{7}Li, resulting in a higher diffusion rate of ^{6}Li due to its 15% mass difference relative to ^{7}Li. However, in these experiments, the dose of Li used, either orally or injected, is about 1 mEq kg^{-1} (the LD_{50} is 15 mEq kg^{-1} [13]), corresponding for a mouse of 30 g with 2.5 ml of blood volume to a blood concentration of about 80 mg L^{-1}. The resulting blood Li level is higher than the natural baseline, which is about 1 μg L^{-1} in mammalian blood,[16] by a factor of 8×10^5, and is higher than the recommended Li blood levels for patients under Li medication (0.7 mmol L^{-1} [17]), by a factor of 16. Therefore, the reported experiments[12–15] that used pure ^{6}Li or ^{7}Li were carried out at Li concentrations that were well above natural levels and are not representative, except for diffusive processes, of biological conditions.

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In the present study, we explore the natural variations of the $^{7}Li/^{6}Li$ ratio in an experimental animal model, sheep. We report the preliminary systematics of $\delta^{7}Li$ in several organs which suggest that Li isotopes do not fractionate during diffusion processes only, but that other processes, which are probably organ-specific or molecule-specific, are also implied in the Li metabolism.

Material

The design of the feeding experiment for sheep is fully described in Zazzo *et al*.[18] A brief summary is given here. Three Suffolk cross lambs (2 males and 1 female), were raised at the Teagasc Grange Beef Research Centre, Dunsany, Co. Meath, Ireland. The experimental diet consisted of 76% (wet weight basis) pelleted maize concentrate and 24% (wet weight basis) maize silage. The animals were kept on the experimental diet for 231 days. The heterogeneity of this diet has been evaluated by measuring three different aliquots. Upon completion of the experiment (April 3rd, 2007), they were transported to the Teagasc, Ashtown Food Research Center, where their organs were excised and immediately freeze dried. Blood was centrifuged in order to separate the serum from the RBC and was freeze dried. All procedures employed in this study were in accordance with EU regulations concerning animal welfare and use. The experiment was carried out with the approval of Teagasc, the Irish Agriculture and Food Development Authority. In the present study, we analyzed the Li isotope composition of the diet, red blood cells, serum, muscle, liver, brain and kidney.

The three samples of human blood were collected at the Etablissement Français du Sang from blood donors (2 females and one male) in compliance with the relevant laws of the Etablissement Français du Sang and the Ecole Normale Supérieure de Lyon.

Method

Sample dissolution and Li separation

Lithium isotope compositions were measured by Multicollector Inductively Coupled Plasma Mass Spectrometry (MC-ICPMS) after dissolution of the samples and Li pre-separation in a clean laboratory.[19,20] In brief, between 100 and 200 mg of samples were first dissolved by microwave digestion using distilled concentrated HNO_3. After evaporation to dryness, samples were dissolved alternatively in concentrated HCl and in aqua regia at 150 °C for 2 weeks, to obtain complete dissolution. All samples were then dried and dissolved in 1.0 M HCl for solid–liquid chromatography. Lithium separation was performed using 8 cm high AG50-X8 cation exchange resin columns.[19–21] Each sample was passed 3 times through the columns in order to fully purify the Li fractions from the matrix.

Mass spectrometry

Lithium isotope compositions of the three blood samples were measured on the Nu Instrument Multicollector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS) at the University of Hawaii, USA (Geology & Geophysics Department), using the techniques described in Vigier *et al.*, 2009.[20] Lithium isotope compositions of all other samples were measured using the Thermo Scientific Neptune *Plus* MC-ICP-MS at CRPG (Université de Lorraine, France). Since the Li fractions covered a large range of concentrations (from <1 ng Li to >100 ng Li), we used two different techniques on the Neptune *Plus*. For the most concentrated samples (typically 2–100 ng), we used the ''wet-plasma'' technique, in which aerosols produced by a PFA micro nebulizer (100 μl min^{-1} in self aspiration) are introduced into the argon plasma *via* a Thermo quartz dual spray chamber (SIS). Here, the nickel X-skimmer and Jet-sampler cones were chosen instead of the ''standard'' cones. For samples containing less than 1 ng Li, we used a Cetac Aridus II™ desolvating system which allowed us to improve the Li signal by a factor of $\sim$10. In this ''dry plasma'' configuration, standard cones from Thermo Scientific were used.

We determined the $^{7}Li/^{6}Li$ ratio of each sample using a sample-standard bracketing technique, where a blank and a standard (L-SVEC pure solution) are measured before and after each sample. This technique is classically used for Li (and other) isotope measurements by MC-ICP-MS and allowed us to correct for the instrumental mass bias that drifted with time at a rate of about 0.1‰ per hour. Each measurement consisted of 30 cycles of 8 s each, where ^{7}Li and ^{6}Li were simultaneously measured on two different collectors. Blanks were systematically determined. They consisted of a 1 min measurement of ^{6}Li and ^{7}Li during aspiration of ultrapure 0.05 M HNO_3, after a cleaning procedure that typically lasted for $\sim$7 minutes. The blank ^{7}Li signal ranged between 3 mV and 50 mV, while the ^{7}Li signal for the samples ranged between 1 V and 4 V. Total procedure blanks (including chemistry) were found to be negligible (<0.1 mV ^{7}Li).

For all samples, 2σ internal errors on the $\delta^{7}Li$ ranged between 0.01‰ and 0.13‰, depending mainly on Li concentration. The accuracy and the external reproducibility were estimated based on four different reference materials: NASW (seawater), JG-2 (granite), Li-7N and Li-6N (lithium solutions), ran at the same concentration as the samples.[22,23] Average $\delta^{7}Li$ values fell within the published range for these materials, and the 2σ reproducibility was determined to be 0.3‰ for the two pure Li solutions, 0.5‰ for seawater and 0.7‰ for JG-2 granite (Table 1). A sample of serum (#S-1) was duplicated 7 times and the 2SD (standard deviation) obtained was 0.3‰.

Results

Results for samples are presented in Table 2. The whole Li isotopic range spans almost 40‰ between kidney (2.0 ± 5.5‰) and muscle (38.7 ± 2.3‰). Red blood cells and serum have slightly different $\delta^{7}Li$ values, 3.3 ± 0.2‰ and 4.8 ± 0.04‰, respectively, which are close to the kidney $\delta^{7}Li$ values. Brain $\delta^{7}Li$ values are similar to those of the intake, 13.4 ± 3.9‰ and 16.8 ± 2.8‰, respectively. Liver is slightly enriched in ^{7}Li relative to the diet with a $\delta^{7}Li$ value of 21.5 ± 2.5‰.

Table 1 Accuracy and external reproducibility (values in italic) of the δ^7Li value on four different reference materials used in the study

		Pure solution		Passed through columns	
	[Li] (ng ml^{-1})	Li-7N	Li-6N	Granite JG-2	Seawater SW
Average δ^7Li measured at UH (Nu 500)	60	30.15	−8.09	−0.52	30.88
		0.61	*0.37*	*0.46*	*0.69*
		n = 26	n = 5	n = 2	n = 8
Average δ^7Li measured at CRPG (Neptune Plus)	8–30	30.21	−8.2	−1.12	31.32
		0.20	*0.33*	*0.3*	*0.77*
		n = 15	n = 6	n = 2	n = 3
	0.6–3	30.43	−8.15		31.57
		0.34	*0.21*		*0.30*
		n = 19	n = 2		n = 4

Table 2 δ^7Li values obtained for three human blood samples (blood *a*, *b* and *c*) and organs from three different sheep (1, 2 and 3). Errors are 2σ. RBC stands for red blood cell, S for serum, Mu for muscle, Li for liver, Br for brain, Kd for kidney, and Di for diet

	δ^7Li	2σ
Blood *a*	6.7	0.05
Blood *b*	6.5	0.04
Blood *c*	6.4	0.04
RBC-1	3.1	0.37
RBC-2	3.4	1.03
S-1	4.8	0.15
S-2	4.8	0.31
Mu-1	40.3	0.03
Mu-3	37.1	0.04
Li-1	19.0	0.08
Li-2	24.0	0.05
Li-3	21.5	0.05
Br-1	17.9	0.04
Br-2	11.5	0.06
Br-3	10.7	0.04
Kd-2	5.9	0.10
Kd-3	−1.9	0.13
Di-1	17.5	0.10
Di-2	18.4	0.01
Di-3	14.5	0.01

The three human blood samples yield δ^7Li values regrouped around 6.5 ± 0.2‰.

Discussion

Our results on the natural variations of Li isotopes in sheep are the first data reported for biological samples of animal origin. Interestingly, the spread of the results (~40‰), while preliminary, already encompasses most of the isotopic variability reported so far for geological samples (~50‰, Fig. 2). However, this must be confirmed on other large mammals, *e.g.* pig. Mice or rats are not suitable animal models because adequate amounts of material for isotopic analysis cannot be obtained due to their small size and the low Li concentration of 1 ng g^{-1} in organs.[16]

Since Li does not seem to bind to a specific protein, ligand-induced fractionation, as for Cu and Zn with histidine and cysteine,[24] is unlikely. Experiments using pure ^{6}LiCl and ^{7}LiCl demonstrated that ^{6}Li is more reactive than ^{7}Li, resulting in kinetic isotopic fractionation in the body.[12–15] While some of our results are consistent with this hypothesis, other results show that kinetic processes alone cannot explain the observed Li isotope systematics in the sheep model. Lithium enters the body during intestinal absorption with an isotopic fractionation compatible with a kinetic process, because the serum δ^7Li value is strongly ^{7}Li-depleted (~10‰) relative to the diet δ^7Li value (Table 1, Fig. 1). Likewise, red blood cells are slightly ^{7}Li-depleted relative to the serum, which agrees with the hypothesis of faster diffusion of ^{6}Li into erythrocytes.[12] With the possible exception of kidney, however, the remaining organs are ^{7}Li-enriched

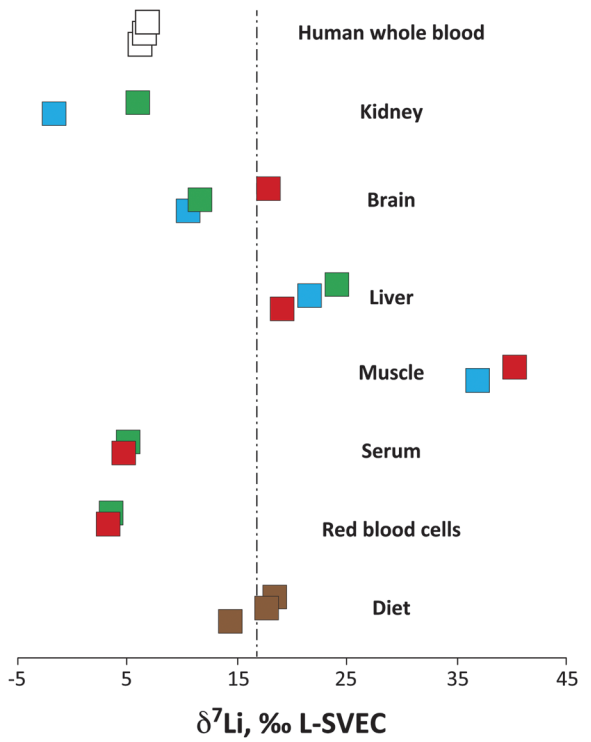

Fig. 1 Distribution of the δ^7Li values obtained for the human blood samples and sheep organs.

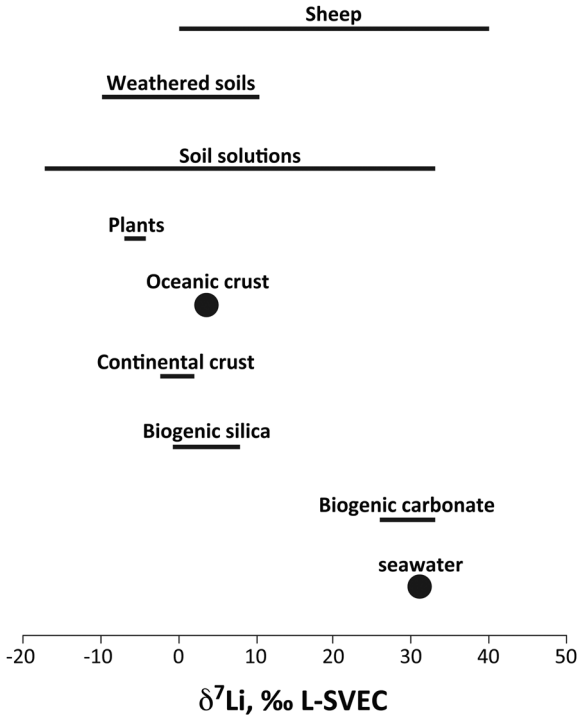

Fig. 2 Distribution of the δ^7Li values obtained for sheep organs and on geological samples. Data from ref. 29.

relative to the serum δ^7Li value (Fig. 1). We have simulated the distribution of the Li isotope compositions in the body of a mammal using a scheme resembling a box-model (Fig. 3). In this oversimplified representation, all the organs have the same size and all the Li fluxes are equal. Blood serum is the central reservoir, from which Li is dispatched to the organs (Fig. 3). All the peripheral organs are either equal to or ^{7}Li-enriched relative to the serum, leaving Li in blood serum isotopically out of equilibrium (Fig. 3). An unknown ^{7}Li-depleted reservoir must account for the observed isotopic pattern in the peripheral organs (Fig. 3). Most of the representative organs, in terms of their relative weights in the body, have been analyzed in the present study, but we lack data for bone and skin, which represent a significant part of the total body weight. Pharmacokinetic models of sheep highlighted the importance of kidney and the very fast excretion of Li in urine.[25] Considering this result together with the possible ^{6}Li-depleted signature of kidney compared to serum, it appears possible that the ^{7}Li enrichment of muscle and liver can be partly balanced by a depletion of ^{7}Li in the kidney. However, it remains to be understood what is the mechanism that drives the δ^7Li of muscle to such high values relative to that of serum.

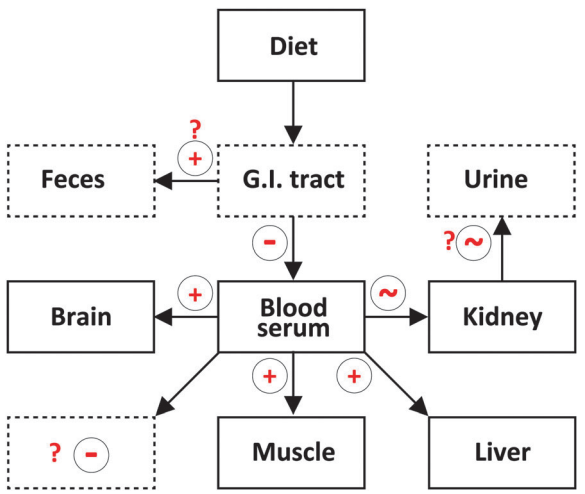

Fig. 3 Schematic box model of Li isotopes in the body, involving reservoirs and fluxes of equal size. The red signs "+" and "−" are for apparent ^{7}Li-enrichment and ^{7}Li-depletion, respectively, while the sign "∼" stands for no apparent isotopic fractionation.

The large difference between the diet and muscle δ^7Li values suggests that the Li isotope composition of bone can serve to reconstruct the mammalian trophic chain, as in the case of other metals like calcium, iron, copper and zinc.[26–28] This should hold whatever the δ^7Li value of bone: the difference between the δ^7Li value of diet and muscle for an herbivore will be transferred up the trophic chain to carnivores, because the δ^7Li value of the herbivore's muscle will fix the δ^7Li value of the carnivore's diet. Theoretically, the Li isotopic difference between the bone of herbivores and carnivores should thus be equal to the Li isotopic difference between the diet and the muscle of herbivores, *e.g.* ∼20‰. In the absence of experimental information, this remains a hypothesis that must be tested using bones of mammals fed on a controlled diet. Moreover, it is unknown whether Li from plant food and animal food will be absorbed with comparable efficiencies and what will be the Li isotopic fractionation associated with both types of food. The measurement of the bone δ^7Li values of mammals living in the same trophic chain would help to answer this question.

Conclusion

We report for the first time variations of natural abundances of Li isotopes in an animal model. The results show that apparently all the fluids and organs measured so far, *i.e.* serum, red blood cells, muscle, liver, brain and kidney, have a characteristic Li isotopic signature. Interestingly, liver and muscle are ^{7}Li-enriched relative to the δ^7Li value of serum, demonstrating that kinetic processes are not the driving mechanisms of isotopic fractionation at least in these organs. This may suggest that other mechanisms, possibly through the binding of Li to yet unidentified molecules, led to the fractionation of Li isotopes. Whatever the origin of the Li isotopic fractionation between organs, the determination of Li concentration in organs will help to better constrain Li fluxes under baseline and therapeutic conditions.

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